Irritection® Assay System

Instruction Manual

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Irritection® Assay System

Instruction Manual

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Executive Summary

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OVERVIEW: OCULAR IRRITECTION ASSAY
SCIENTIFIC BACKGROUND

The proprietary Ocular Irritection assay is a standardized and quantitative *in vitro* test that can be employed to detect, rank, and predict the ocular irritation potential of cosmetics, consumer products, pharmaceuticals, and chemical raw materials. The Irritection test methods are based on the understanding that the corneal irritancy of chemicals is known to be related to their propensity to promote denaturation and disruption of corneal proteins. Consequently, the Irritection methods have been developed as *in vitro* tests that mimic these biochemical phenomena. The rest itself consists of two essential components. The first component is a membrane disc that permits controlled delivery of the test material to a reagent solution. The second component is a proprietary reagent solution that is composed of proteins, glycoproteins, lipids and low molecular weight components that self-associate to form a complex macromolecular matrix. These components are depicted schematically in Figure 1.1.

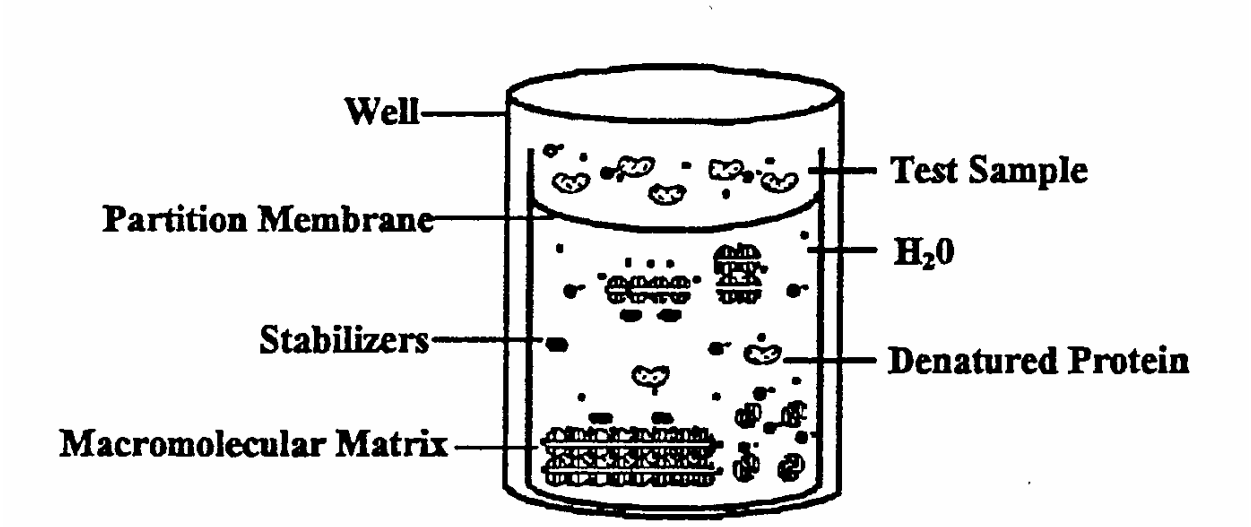

Figure 1.1. The Ocular Irritection Model

When the assay is actually performed, controlled mixing of the test material and the reagent solution during the assay incubation period promotes protein denaturation and disaggregation of the macromolecular matrix. The changes in protein structure that are induced by the test material are then quantified by measuring the resulting changes in turbidity (OD_{400}) of the reagent solution.

Analysis and interpretation of the resulting data is performed with a Windows™ – based computer software program (Chapter 7) that has been developed by InVitro International. This program compares the increase in optical density (OD_{400}) produced by the test material to a standard curve that is constructed by measuring the increase in OD_{400} produced by a set of

Calibration substances. These Calibrators have been selected for use in this test because their irritancy potential has been previously documented in a series of *in vivo* investigations. This approach permits calculation of an Irritection Draize Equivalent (IDE) score. The predicted *in vivo* classification of ocular irritants, based on this scoring system, is shown in Table 1.1.

Table 1.1. Relationship of Irritection Draize Equivalent (IDE) Score to Irritancy Classification for the Ocular Irritection Test Method

Irritection Draize Equivalent (IDE)	**Predicted Ocular Irritancy Classification**
0.0 – 12.5	Minimal Irritant
12.5 – 30.0	Mild Irritant
30.0 – 51.0	Moderate Irritant
51.0 – 80.0	Severe Irritant

APPLICATIONS OF THE OCULAR IRRITECTION ASSAYS

Two different versions of the Ocular Irritection Assays have been formulated. One version provides assessment of a broad range of irritants. This regular version of the test has successfully been employed for the following types of applications: industrial chemicals, cosmetics, petrochemicals, surfactants, and alkaline compounds. A second version of the test, termed the high sensitivity ocular assay, has been specifically developed to permit assessment of the irritancy potential of materials that display very little ocular irritancy. This test is normally employed to assess the irritancy of baby shampoos, certain cosmetics, and other minimal irritants.

As described in Chapter 3, the experimental protocols that are utilized to determine the irritancy potential of these types of compounds generally fall into one of two different categories. Cosmetics, industrial chemicals, and petrochemicals that have a pH $\leq$ 8.5 are most commonly characterized by utilizing them to perform volume – dependent dose-response studies. By contrast, surfactants and surfactant-containing formulations are most successfully characterized by utilizing them to perform concentration-dependent dose-response studies.

The Ocular Irritection assay system provides significant benefits when compared to the *in vivo* Draize test method. The quantitative Ocular Irritection *in vitro* assay has been found to be highly reproducible. Of even greater relevance, the Ocular Irritection assay method can be readily employed to evaluate multiple samples at varying volumes or concentrations. Thus, the test serves as an extremely useful screening tool that facilitates all stages of raw material selection, formulation development, and final product selection.

OVERVIEW: DERMAL IRRITECTION ASSAYS

SCIENTIFIC BACKGROUND

The proprietary Dermal Irritection assay is a standardized and quantitative *in vitro* test that can be employed to detect, rank, and predict the dermal irritation potential of cosmetics, consumer products, pharmaceuticals, and chemical raw materials. This assay, depicted schematically in Figure 1.2 below, is based on the principle that chemicals that cause dermal irritation are known to induce alterations in the structure of keratin, collagen and other dermal proteins. The dermal Irritection Assay System is an *in vitro* test that mimics these biochemical phenomena. Like the Ocular version of the test, the Dermal test also consist of two components. The first component is a membrane substrate that has been modified by covalently crosslinking a mixture of keratin, collagen and an indicator dye to it. The second component is a reagent solution consisting of a highly organized globulin/protein macromolecular matrix.

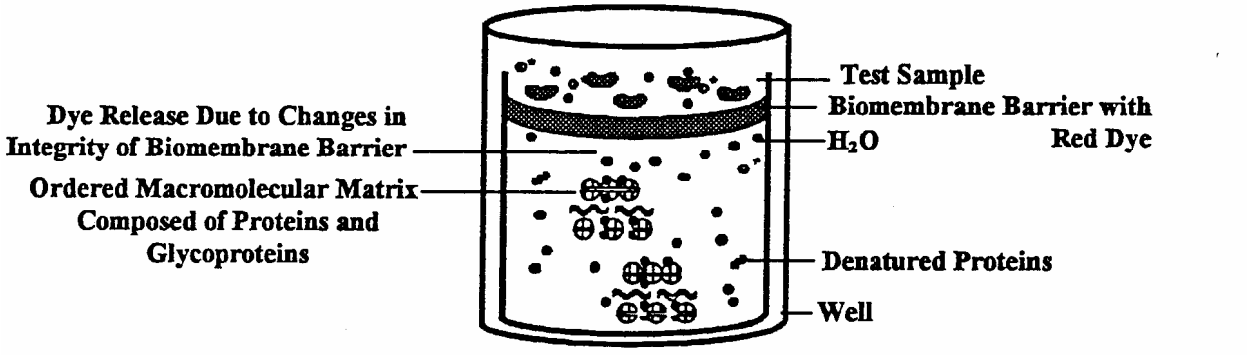

Figure 1.2. The Dermal Irritection Model

To perform this standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane containing a keratin-collagen matrix coated with a dye. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a proprietary solution containing highly-ordered globulins and glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein solution. In addition, the dye that has been dissociated from the biobarrier during transit of the applied sample may be detected spectrophotometrically.

The extent of dye release and protein denaturation may be quantified by measuring the changes in optical density of the reagent solution at 470 nm (OD_{470}). Comparison of these optical density measurements to those produced by standard chemical irritants permits calculation of a Human Irritancy Equivalent (HIE) score that has been shown to be directly related to the potential dermal irritancy of the test material. Analysis of the data and calculation of the HIE score is achieved with the Irritection computer software which includes programs that compare the

increase in optical density (OD_{470}) produced by the test material to a standard curve that is constructed by measuring the increase in OD_{470} produced by a set of Calibration substances. The predicted *in vivo* classification, based on this scoring system, is shown in Table 1.2.

Table 1.2. Relationship of Human Irritancy Equivalent (HIE) Score to Irritancy Classification for the Dermal Irritection Test Method

Human Irritancy Equivalent (HIE)	**Predicted Dermal Irritancy Classification**
0.0 – 0.90	Non-Irritant
0.90 – 1.20	Non-Irritant/Irritant
1.20 – 5.0	Irritant

APPLICATIONS OF THE DERAMAL IRRTECTION ASSAYS

Like the Ocular Irritection assays, the Dermal Irritection assays have been formulated to characterize the irritancy potential of both typical dermal irritants and minimal irritants. The regular version of the test has been successfully employed to characterize anti-irritants, industrial chemicals, cosmetics, petrochemicals, and surfactants. The high sensitivity version of the test has been utilized to characterize industrial chemicals, cosmetics, textile extracts, and other minimal irritants.

The experimental protocols (Chapter 3) that are utilized to determine the irritancy potential of these types of compounds generally fall into one of two different categories. Cosmetics, industrial chemicals, and petrochemicals that have a pH $\leq$ 9.0 are most commonly characterized by utilizing them to perform volume-dependent dose-response studies. Alternatively, surfactants and surfactant-containing formulations are most successfully characterized by utilizing them to perform concentration-dependent dose-response studies.

The Dermal Irritection assay system provides significant benefits when compared to *in vivo* test methods. The quantitative Dermal Irritection *in vitro* assay has been found to be highly reproducible. Of even greater relevance, the Dermal Irritection assay method can be readily employed to evaluate multiple samples at varying volumes or concentrations. Thus, the test serves as an extremely useful screening tool that facilitates all stages of raw material selection, formulation development, and final product selection.

DESCRIPTION OF KIT COMPONENTS

Reagent Powder:	When hydrated, forms a solution containing an ordered macromolecular matrix. Proteins in this solution undergo changes in conformation when exposed to an irritant.
Hydrating Solution:	Employed to rehydrate the reagent powder and facilitate formation of the ordered protein matrix.
Blanking Buffer:	Employed as a control solution which accounts for test sample background contribution to the assay.
Activator (A):	Lowers the pH of the reagent solution to the appropriate level to initiate formation of the ordered macromolecular matrix when the protein reagent has been rehydrated.
Four Calibrators Labeled Cal 0, Cal 1, Cal 2 and Cal 3:	Defined irritants that are employed in each assay to provide standardization and determination of irritancy equivalent scores.
Tow Quality Controls Reagents (QC 1 and QC 2):	Defined irritants that are employed in each assay as quality assurance controls to ensure proper performance of the assay.
Inhibition Check (IC):	A strongly irritating substance that is employed as a positive control to check for false negative results at the completion of each assay.
Membrane Discs:	A semi-permeable membrane that facilitates controlled delivery of the test sample into the protein regent. The Dermal Irritection membranes also incorporate a keratin/collagen matrix that has been corsslinked to a red dye which serves as a component in the overall reaction.

Irritection® Assay System

Instruction Manual

Chapter 2
Scientific Background

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INTRODUCTION

The Irritection assay system consists of newly-developed test kits, instrumentation and computer software that have been integrated to provide an automated *in vitro* testing capability that detects, predicts and ranks the ocular and dermal irritation potential of several types of materials. Because the system is reliable, rapid and cost-effective, it can be readily employed as a broad-screen complement of a complete replacement for more variable, costly and time-consuming *in vivo* test methods. Consequently, the Irritection system is now being used as an efficient tool that substantially reduces the cost of developing products such as cosmetics, petrochemical formulations, surfactant-containing compounds and wide variety of consumer products.

BACKGROUND: BIOCHEMICAL BASIS OF THE IRRITECTION TEST METHODS

The original impetus for development of the Irritection system was the recognition that a need existed to prove *in vitro* test methods that could be reliably substituted for the Draize tests of ocular and dermal irrtancy.[1] Consequently, investigators focused their studies on defining the mechanisms that were responsible for the corneal opacification and skin irritation that was observed in these *in vivo* tests and identifying potential biochemical substitutes for the corneal and dermal proteins that were effected by chemical irritants. These investigations eventually lead to development of the Eytex and Skintex test methods that were the forerunners of current Irritection system.[2]

It is now understood that the Ocular Irritection tests are based on well-recognized biochemical mechanisms.[3] Specifically, it has been demonstrated that the normal cornea is transparent, in large part because it is composed of proteins and carbohydrates that are highly organized. This precise organizational structure permits light to pass easily through the corneal, thus allowing clear vision. When chemical irritants are placed in the eye, they disrupt these organized proteins and produce the corneal cloudiness that is observed while performing the Draize test. The important contribution of InVitro International's scientists has been the discovery that this process can be mimicked *in vitro* when irritants are applied to a unique mixture of proteins and glycoproteins.

The major constituent of InVitro International's proprietary Ocular Irritection protein reagent is an oligomeric protein consisting of 12 subunits. This oligomer has an apparent molecular weight of approximately 320kD. The protein oligomers tend to self-associate to form larger fibrils that are held together by noncovalent forces. The lyophilized Irritection reagent also contains carbohydrates, lipids and low molecular weight components. When the reagent is hydrated with a buffered salt solution, the oligomeric protein combines with other reagent constituents to form an ordered macromolecular matrix that mimics the highly ordered structures of the transparent cornea.

Application of chemical irritants to the Irritection protein reagent produces biochemical changes that are similar to those observed in the cornea. For example, it has been demonstrated that

cationic and anionic surfactants produce both corneal opacification and increased turbidity of the Irritection reagent because these chemicals promote protein denaturation. Additionally, as the proteins become denatured, they unfold and change shape, a process which is termed changing conformation. As either the corneal or the Irritection proteins change conformation, they begin to disrupt the highly organized matrix structures that surround them. As this process proceeds, the proteins and matrix constituents gradually begin to form minute insoluble particles that re readily observed because the normally clear protein solutions gradually become cloudy. Figure 2.1 depicts these types of changes schematically.

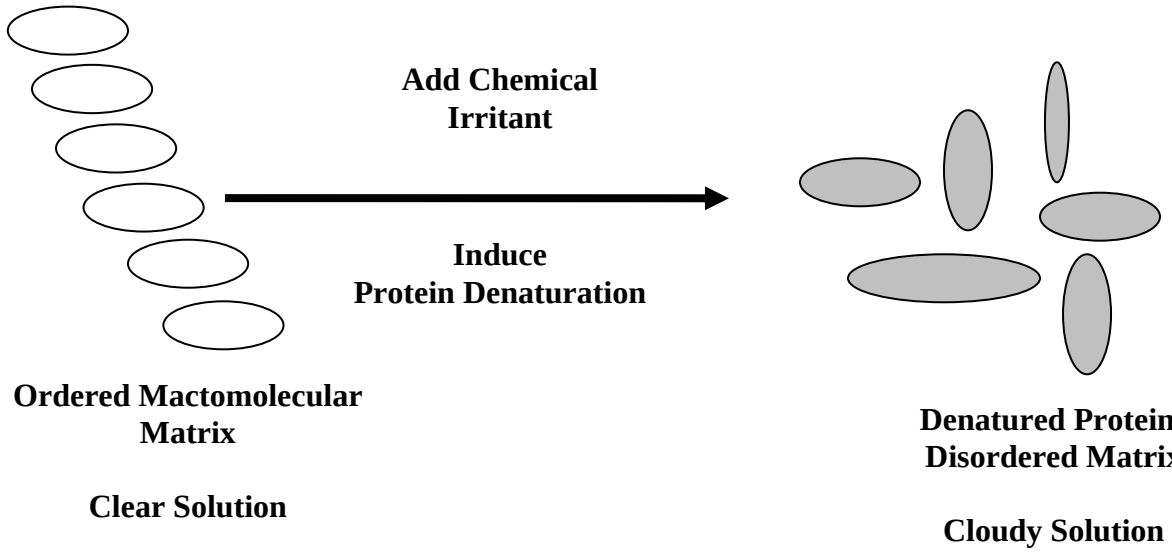

Figure 2.1. Schematic Diagram Depicting Changes in the Irritection Protein Reagent that are Induced by Chemical Irritants

These phenomena can be readily quantified by measuring the changes in light scattering that occur as the protein matrix becomes disrupted and the turbidity of the solution increases. In the Irritection system, this increase in light scattering is detected and quantified at a wavelength of 400 nm (OD_{400}) with a platereader spectrometer that has been specifically adapted for this purpose.

Normally, as the concentration or volume of the tested irritant substance is increased, the Irritection protein reagent becomes more denatured and the amount of scattered light gradually increases until a maximal optical density is produced when all the proteins have become altered. As a result, chemical irritants typically produce a linear or sigmoidal dose-response curve when tested in the Irritection system. An example of such volume-dependent dose-response curve is shown in Figure 2.2 on the next page.

By contrast, surfactants commonly produce a slightly different type of dose-response curve when analyzed with the Irritection system. To understand this phenomenon, it is important to recognize

that surfactants function to solubilize organic substances in water because they are comprised of a hydrophobic "tail" and a hydrophilic "head". When a surfactant "dissolves" an oil in water, it does so because its "tail" portion is soluble in the oil droplet and its "head" is soluble in water. When surfactants are analyzed with the Irritection system, they produce a bell-shaped does response curve that results from the fact that small quantities of surfactant will denature and disrupt the protein matrix to produce the expected increase in turbidity. However, as the amount of surfactant in the assay increases, the denatured proteins, which are organic molecules, are dissolved just as oil droplets would be. When this occurs, the opaque protein reagent is clarified and, as shown schematically in Figure 2.2, the OD_{400} decreases.

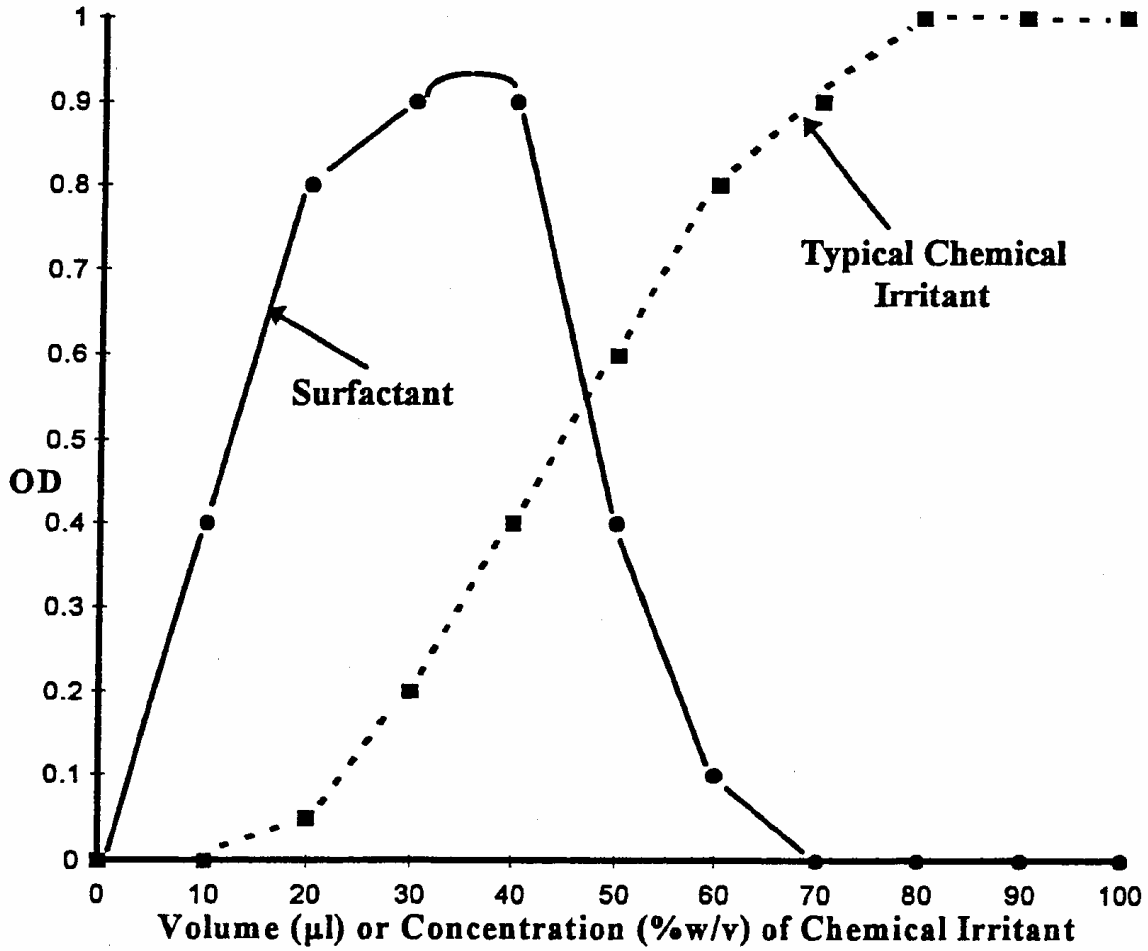

Figure 2.2. Schematic Diagram Depicting the Dose-Response Curves Produced by Surfactants and Typical Chemical Irritants

Chemical irritants and surfactants produce the most controlled and reproducible denaturation of proteins when they are gradually added to them. In the Ocular Irritection system, gradual addition of the test substance to the Irritection reagent is achieved by utilizing a membrane disc

of defined porosity as a delivery tool. Therefore, to analyze a substance with the Ocular Irritection system, the test substance is applied to the membrane delivery disc where it gradually diffuses through the porous membrane to mix with the protein reagent for a defined period of time. During this incubation period, the protein matrix undergoes the types of alternations described above. When the end-point has been reached, these changes are quantified by spectrometric methods and the results obtained with the test substance are compared with those obtained with a series of standard compounds that were analyzed under identical conditions. Incorporation of these calibration standards, whose irritancy potential has previously been defined by the Draize method, permits construction of a standard curve that directly relates the optical density measurement of the Irritection test to the maximum 24 hour Draize score determined by *in vivo* testing. An example of this type of standard curve is shown below in Figure 2.33

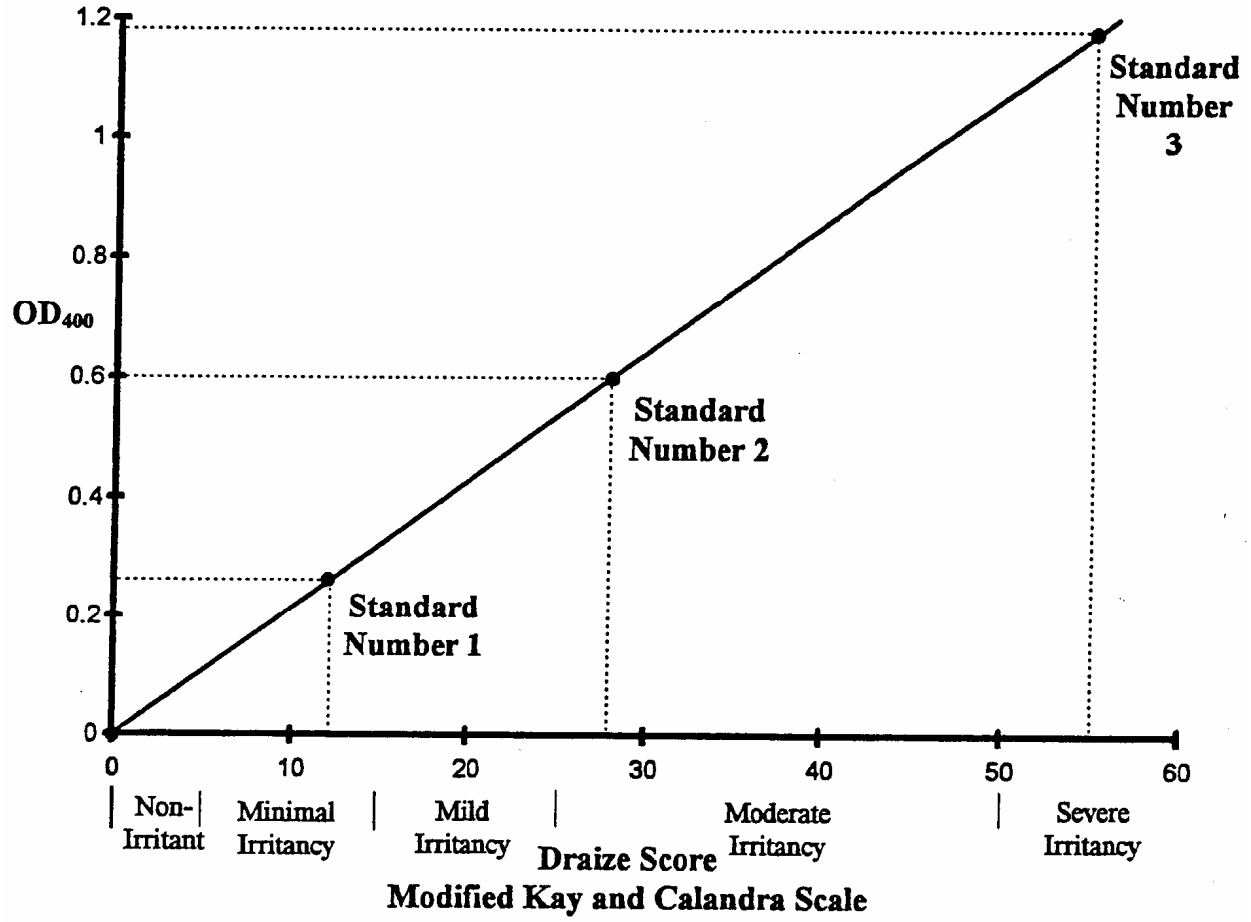

Figure 2.3. An Example of a Standard Curve Demonstrating the Correlation Between Irritection Optical Density Measurements (OD_{400}) and Draize Scores

The results of these types of comparative studies have been utilized to develop what is termed the "Irritection Draize Equivalent (IDE)" score, i.e., a value derived from the *in vitro* studies that

is equivalent to the predicted *in vivo* irritancy score. An example of the comparison of these types of *in vitro* and *in vivo* scores for the Ocular test is shown below in Table 2.1.

Predicated Ocular Irritancy	**Irritection Score (IDE) Range**	**Draize Score Range**
Non-Irritant		0 – 5
Minimal	0 – 12.5	>5 – 15
Mild	>12.5 – 30	>15-25
Moderate	>30 – 51	>25 – 50
Severe	>51	>50

Table 2.1. Summary of the Relationship of Irritection and Draize Scores to Predicted Ocular Irritancy

These type of derived scoring systems have proven to be very useful for facilitating inter-laboratory comparative studies of different types of chemical and chemical formulations.

The Dermal Irritection assay system, which is the enhanced version of the earlier Skintex test method, is also based on the principle that chemical irritants of the skin may be detected because they promote changes in relevant macromolecules and protein denaturation. However, this test method, which is depicted schematically in Figure 2.4, duffers significantly from the Ocular Irritection test in three ways. First, the synthetic biobarrier membrane of the Dermal test

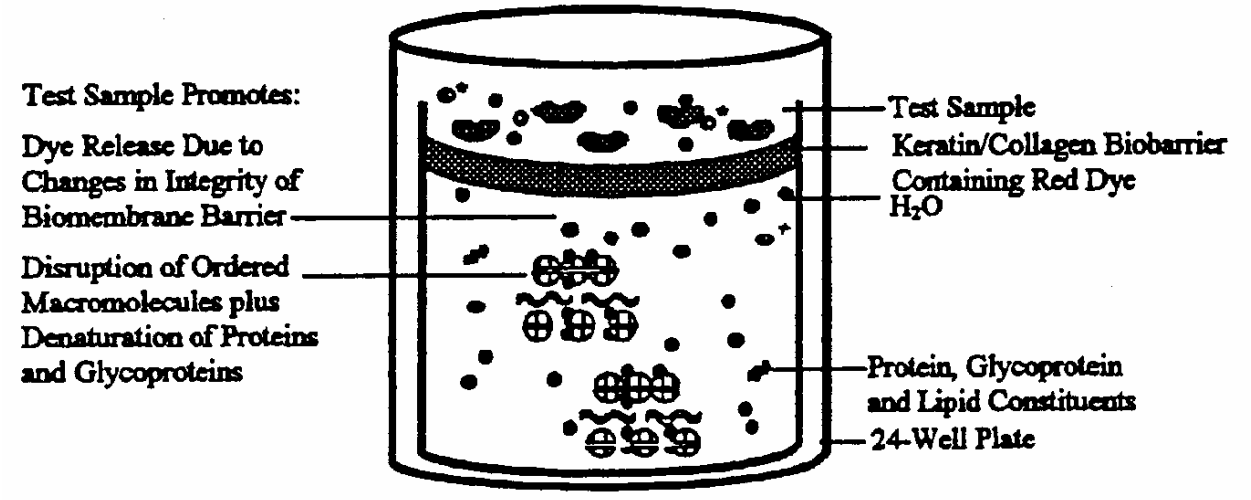

Figure 2.4. Schematic Diagram Depicting The Dermal Irritection Assay System

is somewhat more complex than the membrane delivery system of the Ocular test. Specifically, the Dermal biobarrier consists of a cellulosic membrane that has been coated with keratin and collagen in order to more closely duplicate the normal barrier properties of the dermis. Additionally, during the manufacturing process, a red dye is bound to these proteins. When the test is performed, release of this dye from the protein matrix by the test substance serves to

indicate disruption of the keratin/collagen biobarrier. Second, the composition of the protein mixture found in the Dermal test differs somewhat from that of the Ocular test in the structure of these proteins and dye release are quantified by measuring the absorbance at 470 nm (OD_{470}) rather than at 400 nm as in the Ocular test.

EVALUTION OF THE IRRITECTION TEST METHODS

Extension investigations have been conducted to confirm the accuracy and reliability of both the Ocular and Dermal Irritection test systems. Most studies have focused on demonstrating that the earlier Eytex and Skintex versions of these tests provide results that correlate with *in vivo* findings. Two different approaches have been employed to confirm this correlation. In some cases, the data has been analyzed by performing a linear regression analysis. However, in most instances, because both the *in vitro* methods and the Draize methods do display inherent variation, concordance analysis, performed according to accepted statistical methods,[4,5] has been employed to compare the *in vitro* and *in vivo* test results.

As shown in Table 2.2, initial internal investigations conducted with a wide variety of different types of materials demonstrated a strong correlation between the results of the original Eytex test and Draize score.

Product Class	N	Equivalence	Sensitivity	Specificity	Predictive Value
Consumer Household	54	92%	94%	100%	100%
Consumer Personal	56	93%	95%	100%	100%
Cosmetic Products	34	97%	83%	100%	100%
Industrial Chemicals	67	96%	93%	100%	100%
Industrial Products	102	96%	97%	96%	95%
Pharmaceuticals	39	92%	100%	100%	100%
Food Products	36	87%	84%	91%	84%
Total	388	94%	95%	97%	98%

Table 2.2. Comparison of Ocular Irritancy Predicted by *In Vitro* and *In Vivo* Test methods: Summary of Concordance Analysis

Similarly, investigations performed with the Skintex test, which was the forerunner of the current Dermal Irritection assay system, demonstrated a high degree of correlation with *in vivo* findings. The results of these studies are summarized below in Table 2.3.

Product Class	N	Equivalence	Sensitivity	Specificity	Predictive Value
Consumer Household	84	85%	87%	81%	88%
Cosmetic Products	238	95%	93%	96%	94%
Industrial Chemicals	54	92%	93%	80%	95%
Pharmaceuticals	33	85%	82%	88%	87%
Total	409	92%	93%	88%	89%

Table 2.3. Comparison of Dermal Irritancy Predicted by *In Vitro* and *In Vivo* Test methods: Summary of Concordance Analysis

Subsequent studies conducted in collaboration with the FRAME Alternatives Laboratory at the University of Nottingham Medical School provided similar results. For these investigations, the irritancy potential of 53 different commonly-occurring laboratory chemicals was evaluated with the Eytex test method. The *in vitro* test was shown to have an equivalency of 88%, sensitivity of 92%, specificity of 89%, and predictive value of 89%.

Published scientific studies have confirmed the value of the Eytex version of the Irritection test methods. For example, in a study of 78 petrochemicals that had previously been evaluated with the Draize test, all 22 irritants were correctly classified as irritants by the Eytex method.[6] Additionally, 54 of 56 non-irritants were also correctly classified. There were no false negatives and only two false positives in the data set. The equivalence between the *in vitro* test and the Draize test was found to be 89% and the predictive value of the Eytex test was observed to be 92%. A linear correlation analysis of the data provided a correlation coefficient of 0.76 indicating that there was a significant correlation between the *in vitro* and *in vivo* test methods.

The Eytex test has also been extensively evaluated in a double-blind study of 465 cosmetic product formulations and raw ingredients.[7] the results of these studies are summarized in Figure 2.5.

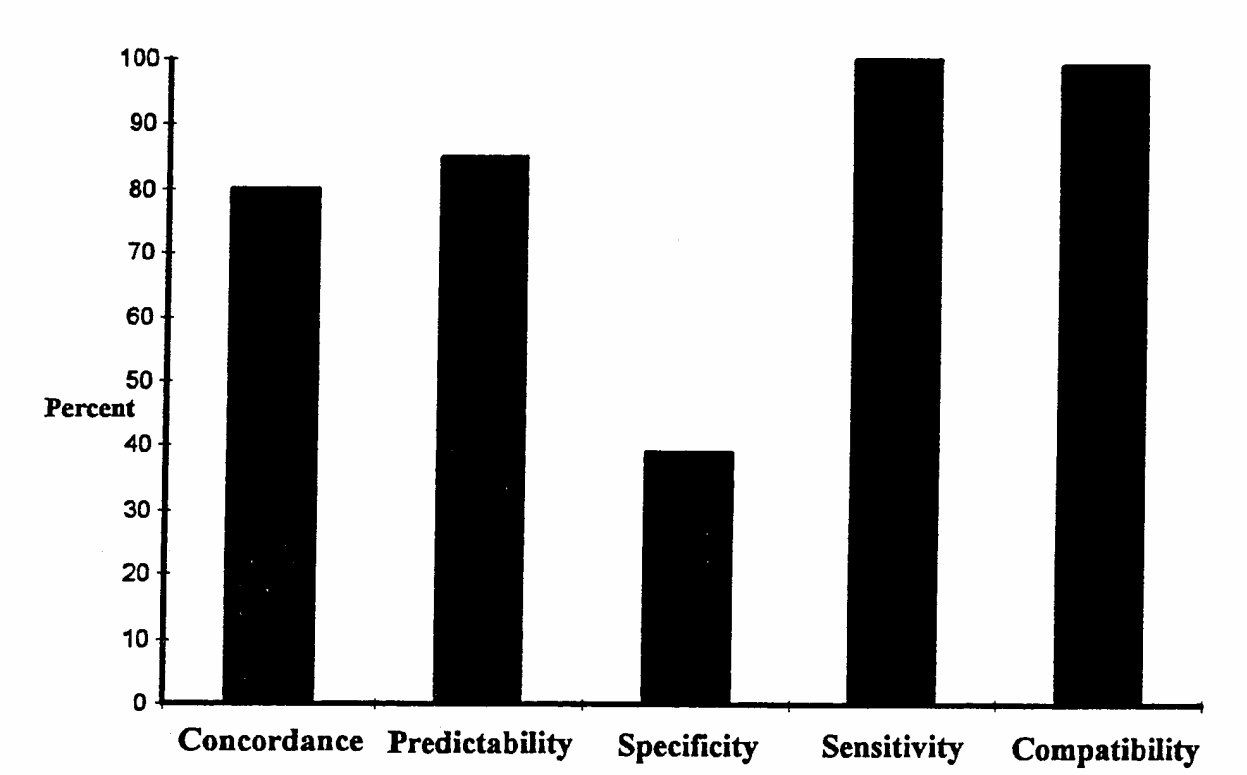

Figure 2.5. Summary of Statistical Parameters Obtained from a Study Of 465 Cosmetics and Raw Ingredients

These findings obtained with over 30 different product types representing a wide range of irritancy clearly demonstrated that the Eytex test results were correlated with rabbit eye irritation data. A strong positive agreement on the *in vitro* and *in vivo* assay results was demonstrated by an overall concordance of 80%. Additionally, the finding of 100% sensitivity and 85% predictability indicated that the *in vitro* test effectively identified irritant cosmetics and their constituents. A compatibility rate of 99% demonstrated that the *in vitro* test was well-suited to accommodate a wide variety of different materials.

The Eytex test has also been applied to assessing the irritancy potential of pharmaceutical intermediates.[8] In this study of 37 test materials which were chosen to represent a broad range of pH, solubility and *in vivo* irritation potential, the Eytex test was demonstrated to have a concordance of 72.2% with the *in vivo* test. By contrast, these authors concluded that *in vitro* tests based on cellular and/or bacterial cytotoxicity end points did not correlate well with the *in vivo* data.

Another recent study of 142 chemicals and chemical products conducted in eight different laboratories clearly demonstrated the correlation of the Eytex test results with those obtained by the Draize method.[9] These investigations confirmed earlier observations that nearly all (93%) of the chemicals tested were compatible with the *in vitro* test method. Repeatability (6.3%) and reproducibility (8.9%) were found to be quite acceptable. More importantly, linear correlation analysis, as show below in Figure 2.6, demonstrated a statistically significant correlation (student's t and Fisher's test $p<0.001$) of the *in vitro* test results with the maximal 24 hour Draize score results. The sensitivity, specificity, and equivalence of the Eytex test were noted to be 94%, 89%, and 78% respectively.

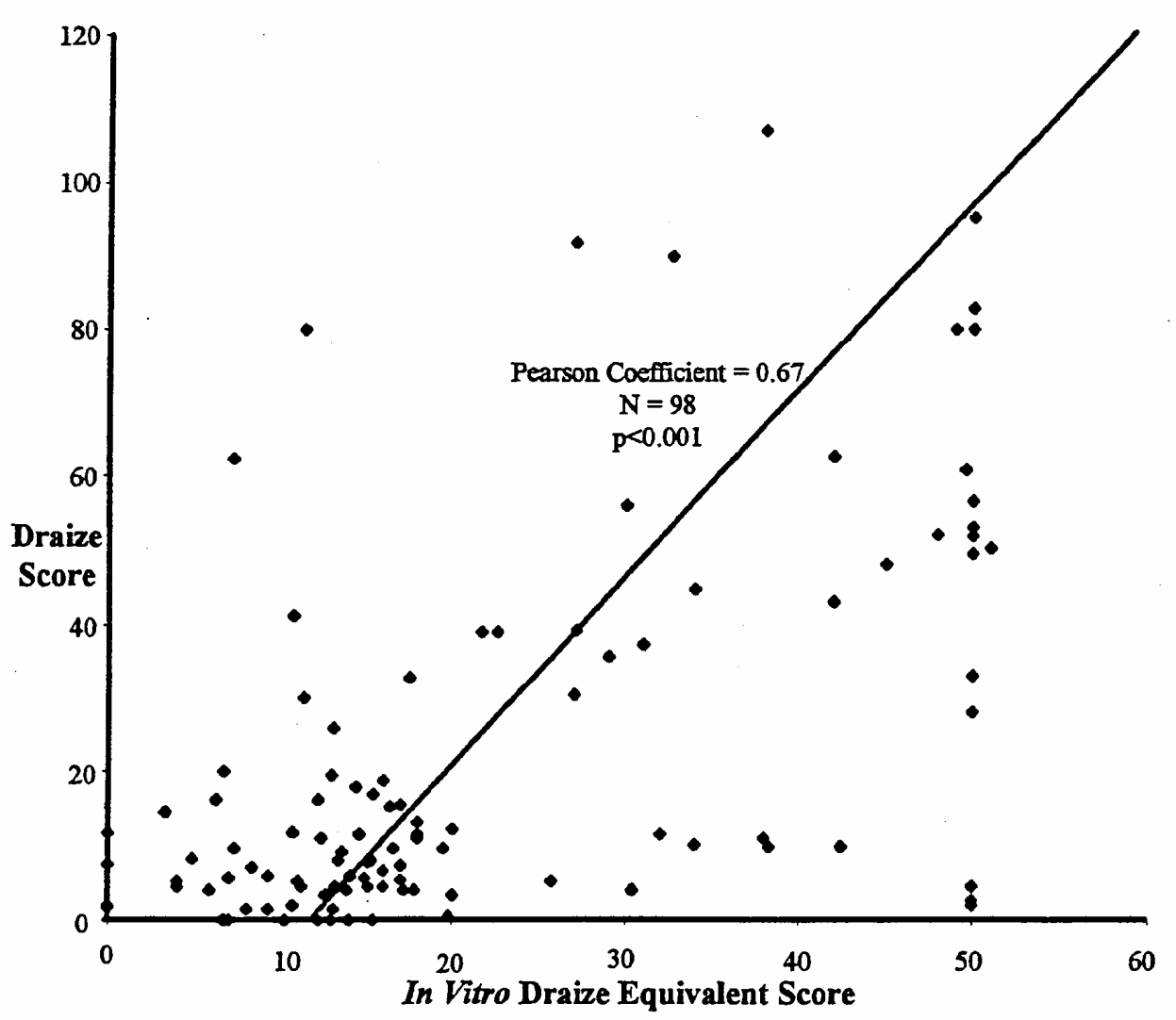

Figure 2.6. Correlation of *In Vitro* Draize Equivalent Scores with Maximal 24 Hour Draize Scores

These authors concluded that the *in vitro* test method "exhibited the characteristics of a good screening method: compatibility with a large range of chemicals; a simple and rapid procedure; good laboratory and inter-laboratory reproducibility; coast effectiveness; high sensitivity, specificity and predictive value; and an low incidence of false negative and false positive results."

And important aspect of the development of the new Irritection test method was confirmation that this new test correlated well the previous Eytex test. This was achieved by utilizing four different laboratories to perform an inter-laboratory comparison of the two methods. As shown in

Figure 2.7, when the mean Draize equivalent scores obtained for the Irritection and Eytex tests were compared, the correlation between the two test methods was excellent.

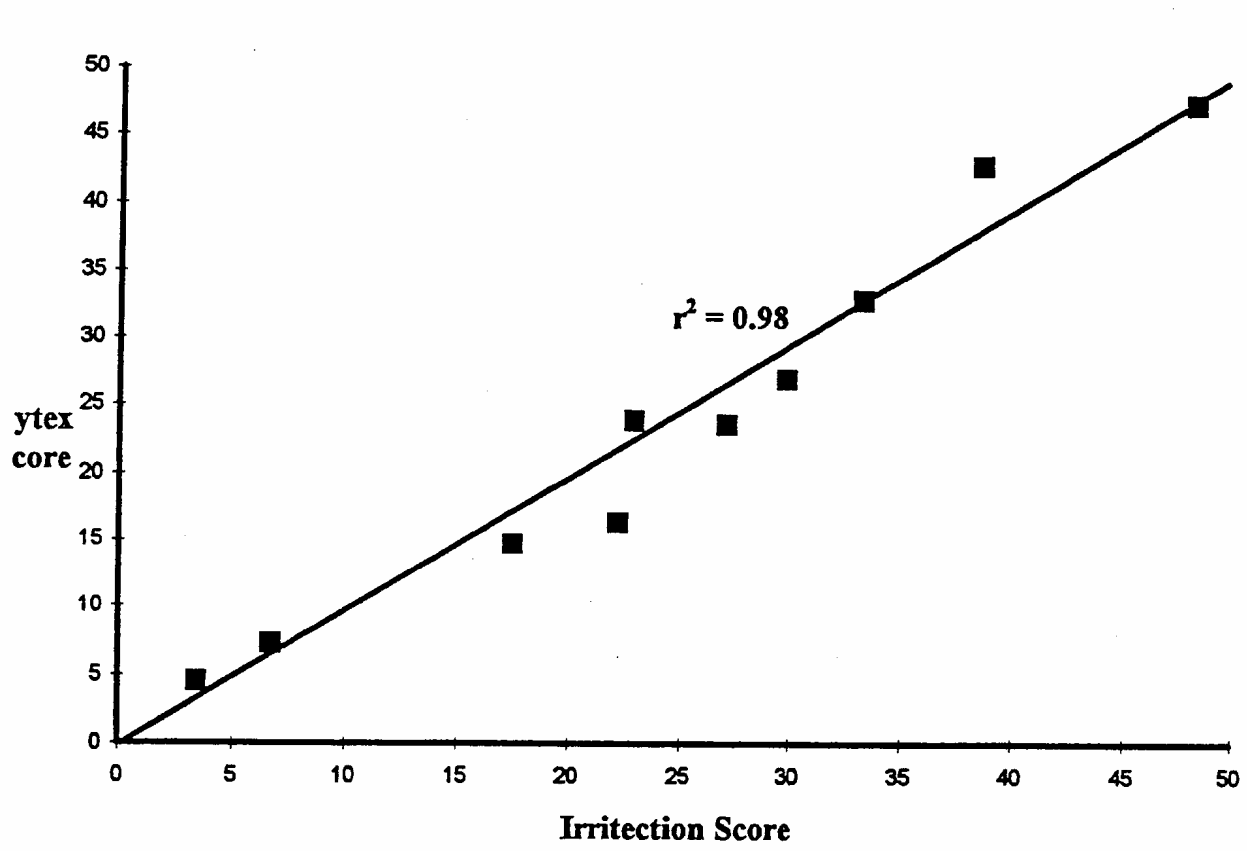

Figure 2.7. Comparison of Mean Eytex and Irritection
Draize Equivalent Scores Obtained for 10 Reference Chemicals

The two tests were also found to be similar with regard to reproducibility, with the Irritection test displaying a coefficient of variation of 12% and the Eytex test 9%.

Taken together, these evaluations of the earlier Eytex and current Irritection systems clearly demonstrate the value of this test method as a reliable and reproducible means of providing both a numerical score and predicted irritant classification of a wide variety of chemicals, raw materials, cosmetics, and pharmaceutical intermediates. These *in vitro* tests display a good correlation with the *in vivo* data whether linear correlation or concordance analysis was utilized to predict irritancy class. The data suggests that concordance analysis provides a somewhat better correlation than linear regression analysis. This is to be expected because these *in vitro* test systems were specifically designed to predict irritancy classifications rather than provide absolute values. However, it is also clear that, for similar chemical formulations, the Irritection test is sufficiently sensitive and reproducible to allow accurate comparisons and rank-ordering of different formulation variations.

SUMMARY AND CONSLUSIONS

In Vitro International's newly-developed Irritection test system is an advanced *in vitro* test that can be employed to predict the irritancy potential of diverse chemicals, raw ingredients and consumer products. The performance of the Irritection system has been significantly enhanced when compared to the earlier Eytex and Skintex versions of this test. The advantages of the current test system include: increased reliability, convenience, and coast-effectiveness. In addition, the Irritection test is performed on instrumentation that is both more reliable and more flexible than that employed previously. The Irritection test also exhibits the reproducibility, sensitivity and specificity that is required of a valid *in vitro* method.

The Irritection test may be used either alone or in combination with other tests for a wide variety of applications. Because the assays are broadly applicable, the Irritection system is best suited to serve as a screening tool that can be employed to facilitate formulation and product development activities. The test method possesses sufficient specificity and sensitivity to permit identification and rank ordering of potential irritants so these may be eliminated from consideration very early in the formulation process. Furthermore, the rapidity and cost-effectiveness of the Irritection assays in this setting make them an excellent replacement for animal testing in the early phases of the product development cycle.

The Irritection tests are also well suited for both the initial and final phases of product development. For example, there is clear evidence that either the ocular or the dermal versions of the test may be employed as a quality assurance test method that serves to define the irritancy specifications for raw materials and other ingredients. This is particularly true if the materials are composed of complex mixtures of natural products that are not easily characterized by other analytical methods. The Irritection tests have also been utilized to confirm the safety of final product formulations, particularly in the cosmetics industry where adequate data bases have been compiled to support this application.

The current application-specific Irritection products have been specifically designed to meet the needs of formulation and product development groups in a wide variety of different industries. These applications are summarized in Table 2.4.

Ocular Assays	**High sensitivity Ocular Assays**	**Dermal Assays**	**High Sensitivity Dermal Assays**
Industrial Chemicals	Baby Shampoos	Anti-Irritants	Industrial Chemicals
Cosmetics	Minimal Irritants	Industrial Chemicals	Cosmetics
Petrochemicals	Cosmetics	Cosmetics	Minimal Irritants
Surfactants		Petrochemicals	Textiles
Alkaline Compounds		Surfactants	

Table 2.4. Current Irritection Assay Applications

In addition to these existing products, In Vitro International's Customized Technology Services group is continually working with customers to develop and refine new applications of the core technology that are specifically designed to meet their requirements.

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Irritection® Assay System

Instruction Manual

Chapter 3
Experimental Protocols

InVitro International
17751 Sky Park East, Suite G
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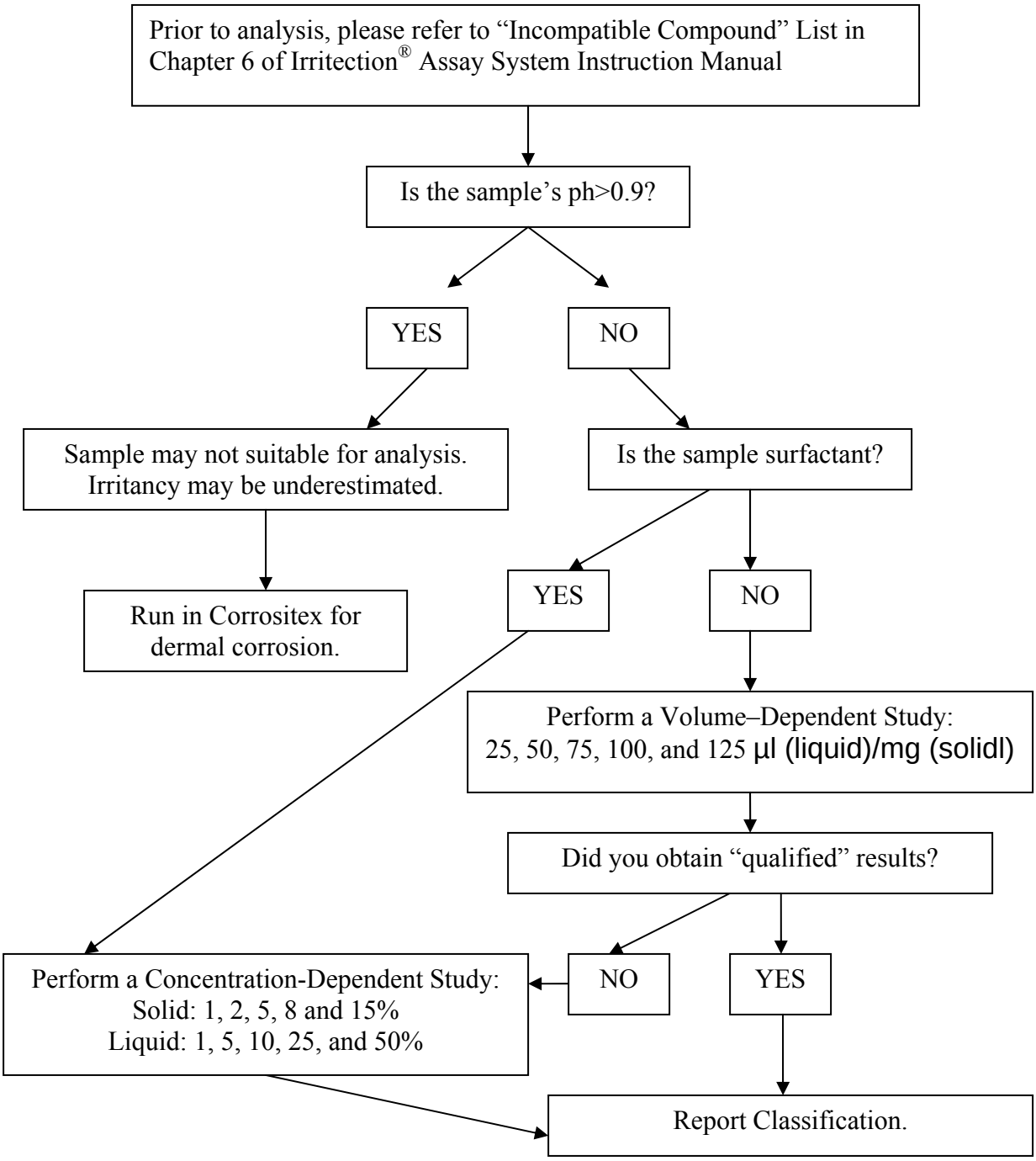
Irritection® Assay System Decision Tree



OVERIVEW

This chapter of the Irritection Assay System Instruction Manual contains four separate assay protocols. These experimental protocols provide a detailed description of the experimental methods that are routinely employed to analyze test samples in both the Ocular and Dermal versions of the Irritection test kits.

As a general rule, most chemicals, petrochemicals, and cosmetics are best characterized by performing a volume-dependent does-response study. Detailed descriptions of these types of experimental protocols may be found on pages 3 through 26 of this chapter. By contrast, because of the phenomena described in the previous chapter, surfactants and surfactant-containing material are best characterized by utilizing concentration-dependent dose-response protocols. Detailed description of these types of experimental protocols may be found on pages 27 through 50 of this chapter.

As noted in Chapter 6 of this manual, extremely alkaline compounds may not be well suited for analysis with these test kits. If the pH of the test sample is >9.5 to 10.0, the sample should be partially neutralized with HCI prior to analysis.

Abbreviated versions of these protocols have been prepared for each specific application of the Irritection Assay System and are included with each specific assay kits. Additional copies of these procedural summaries may be obtained contacting customer service at 1-800-2-INVITRO.

OCULAR RESPONSE ASSAY
VOLUME-DEPENDENT DOSE-RESPONSE PROTOCOL
(DRAIZE EQUIVALENT SCORING SYSTEM)

In Vitro International's Ocular Response Assay is an *in vitro* assay method that may be employed to determine and predict the ocular irritation potential of non-surfactant based chemical, and cosmetic samples. The results of this test have been shown to correlate with those obtained with the *in vivo* Draize test. Ocular Response findings may be related to the *in vivo* test results through the Irritection Draize Equivalent (IDE) scoring system that has been developed for this purpose.

This assay protocol provides a detailed description of the experimental procedures that are required to perform the Ocular Response Assay. The protocol consists of five steps:

1. Preparation of the samples
2. Preparation of the Ocular protein reagent solution
3. Set-up of the assay
4. Incubation for 24 hours to permit reaction of the samples and protein reagent.
5. Determination and interpretation of the assay results.

The first three steps may be completed in approximately two hours. The final step, to be performed on the following day, may be completed in approximately one hour.

Prior to beginning the assay, place the Hydrating Solution in a 25˚C incubator for 1 to 2 hours to ensure that it is approximately 25˚C. Remove the 24-well assay plates filled with membrane discs from the refrigerator. Allow the membrane discs to equilibrate to room temperature (approximately 30-45 minutes) before being used in Step III.D.3.

I. Instrumentation, Reagents and Materials

A. Kit Contains:
   1. Ocular Reagent Powder (1 Bottle)
   2. Ocular Hydrating Solution (1 Bottle)
   3. Ocular Blanking Buffer (1 Bottle)
   4. Ocular Inhibition check (1 Vial)
   5. Ocular Activator (1 Vial)
   6. Four Calibrator Solutions: Cal 0, Cal 1, Cal 2, Cal 3
   7. Two Quality Control Solutions: QC1, QC2
   8. 24-well Assay Plates Filled with Membrane Discs (48 clear discs)–**store at 2-8˚C (2)**

9. Wooden Stirring Sticks (12)
10. Whatman #1 Filter Paper, 12.5 cm diameter (1)
11. Procedural Summary
12. Range Specification Data Sheet
13. Irritection Software Instructions

B. Additional Materials Required:
1. Plate Reader (such as Modified Cambridge 7520 Microplatereader)
2. IBM compatible PC with Irritection Software
3. Incubator Maintained at 25˚C ± 1˚C
4. Balance (110 g Capacity)
5. pH Meter
6. Vortex Mixer
7. Positive Displacement Micro Pipettor (such as Labindustries Popette™ Micropipettor Model P-250, 5 – 250 µl)
8. Multichannel Pipettor (such as Matrix 6 Channel EXP Impact Pipettor 15-1250 µl)
9. Disposable Reservoir Trays (2—such as 100 ml Matrix Disposable Reagent Reservoir)
10. 100 ml Graduated Cylinder
11. 100 ml Beaker
12. Funnel (such as Oxford Vented Disposable Polystyrene Funnel, 12.5 cm diameter)
13. Plastic Forceps
14. Plastic Wrap

II. Sample Preparation

A. Sample Preparation

Typically, five samples may be analyzed at four volumes (50, 75, 100, 125 µl).

1. If the sample is a liquid, dilute the sample to the desired concentration for analysis.
2. If the sample is a solid, attempt to convert in to a liquid by gently warming it. If the sample melts with warming, apply the liquid molten following procedures detailed in Step III.D.3.
3. If the sample is a solid that does not readily melt with warming, weigh out 50, 75, 100, and 125 mg of each sample and place each amount directly into the membrane discs. (if you choose to evaluate 4 samples at 5 volumes, 25 mg should be used as the fifth weight for analysis.) This should be completed prior to rehydrating the Reagent as described in Section III.A below.

III. Procedures

A. Ocular Reagent Preparation:

1. Rehydration:
    a. Remove the Hydrating Solution from the 25˚C incubator.
    b. Using a funnel, pour the entire contects of the Hydrating Solution into the Reagent Powder bottle.
    c. Gently swirl until all of the Reagent Powder is dissolved.
    d. Let the dissolved Reagent stand at room temperature for approximately 10 minutes before filtering.

2. Filtration:
    a. Fold the filter paper and place in funnel.
    b. Pour all of the dissolved Reagent into the funnel and collect the filtrate in a graduated cylinder at atmospheric pressure (do not use a vacuum pump to collect filtrate). This will take approximately 10 minutes.
    Note: During the filtration process, label the 24-well assay plates (Step III.B).
    c. Pour 40 ml of the filtered Reagent into a 100 ml beaker.
    Note: The Reagent must be used within 30 minutes of rehydration.

3. Activation:
    a. Record the initial pH and temperature of the filtered Reagent, verifying that it falls within the following specified ranges: **pH: 7.91-8.19 and T: 23-25˚C.**
    b. Using the positive displacement pipettor, slowly add 800 µl of the Activator to the filtered Reagent. Gently swirl to mix.
    c. Record the final pH of the Activated Reagent solution, verifying that it falls within the following specified range: **pH: 6.42-6.74.** Note: If the pH does not fall within the specified range, recalibrate the pH meter, gently swirl the Reagent and remeasure the pH. If the pH is still not within the specified range, contact your technical representative at 1-800-2-INVITRO.
    d. Using the positive displacement pipettor, slowly add 600 µl of the Activator to the bottle of Blanking Buffer. Gently swirl to mix.
    e. It is not necessary to measure the pH or temperature of the Activated Blanking Buffer.

B. Labeling the 24-Well Assay Plates:

1. Remove the membrane discs from the assay plates and place them in a container. These discs will be used in Step D.1
2. Align the assay plates with the notched corners toward the left and label their lids. A typical plate configuration that has been employed to evaluate five different samples at four different volumes is shows in Figure 3.1 on the next page.

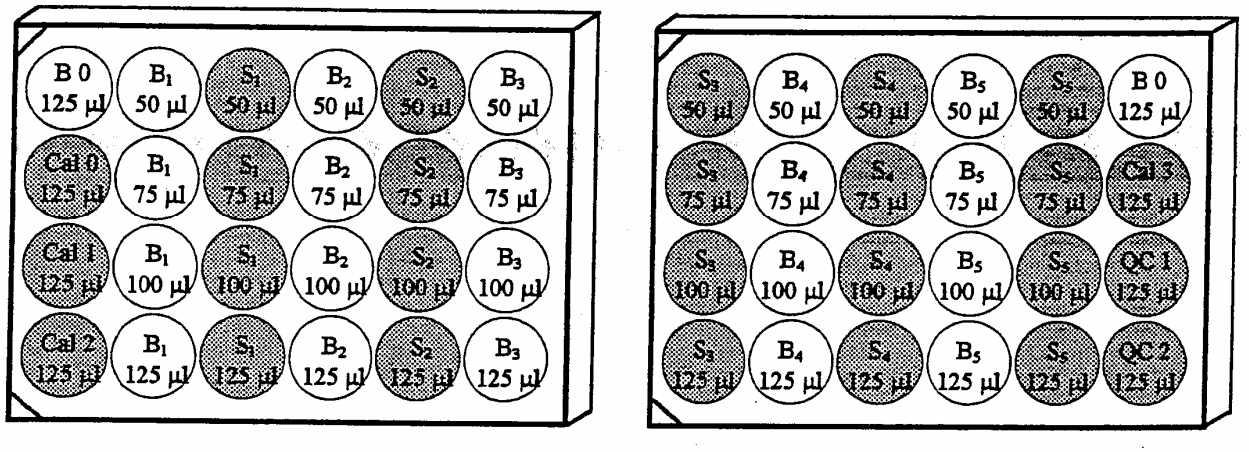


Figure 3.1. Suggested Plate Configuration: Analysis of Five Samples at Four Different Volumes

Figure Legend:

= well contains Blanking Buffer = well contains Reagent

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 50, 75, 100 and 125 µl/mg of sample applied to membrane discs.
S: 1.25 ml Activated Reagent in well, 50, 75, 100 and 125 µl/mg of sample applied to membrane discs.

Note: Alternative assay schemes are shown in Figure 3.3 and 3.4 on pages 14 and 15 of the this chapter.

C. Addition of Reagent and Blanking Buffer to 24-Well Assay Plates:

1. Remove the labeled lid on each assay plate and use the lid or Figure 1 as a reference when filling the wells.
2. Set the multichannel pipettor at 1250 µl.

3. Pour the Activated Reagent into a disposable reservoir tray.
4. Using the multichannel pipettor, add 1250 µl of the Activated Reagent to the following wells: Cal 0, Cal 1, Cal 2, Cal 3, QC 1, QC 2 and all wells designated for analysis of the samples (S).
5. Pour the Activated Blanking Buffer into a disposable reservoir tray.
6. Change pipette tips and add 1250 µl of the Blanking Buffer to the wells designated B 0 on both plates and all other wells designated as blanks (B).

D. Addition of Calibrators, Quality Controls and Samples:

Note: Avoid cross contamination among the separate Calibrator, Quality Control and sample wells by utilizing a clean pipette tip for each.

1. Pipette 125 µl of Cal 0 into a membrane disc and insert it into the B 0 well. Repeat this procedure for the B 0 well of the second assay plate,
2. Pipette 125 µl of Cal 0, Cal 1, Cal 2, Cal 3, QC 1 and QC 2 into the membrane discs and insert them into the appropriate wells of the assay plates.
3. If the sample is a liquid, pipette 50, 75, 100 and 125 µl of each test sample into the membrane discs and insert them into the corresponding blank and test sample wells of the assay plates. (If you wish to evaluate 4 samples at 5 volumes, 25 µl should be used as the fifth volume for analysis.)
4. If the sample is a solid, insert the membrane discs which contain 50, 75, 100 and 125 mg of each test sample into the corresponding blank and test sample wells of the plates.

E. Incubation:

1. Tightly wrap each assay plate with plastic wrap. Place a lid on top of each wrapped plate.
2. Record the technician's name, date and time on the lids of the assay plates.
3. Place the assay plates in an incubator maintained at 25˚C ± 1˚C for 24 hours (± 30 minutes).

F. Removing Membrane Discs:

1. Remove the assay plates from incubator.
2. Remove the lids from the assay plates and save them. They will be used as a reference when transferring the Reagent and the blanking buffer to the clean 96-well reading plate.
3. Carefully remove the plastic wrap from each assay plate and use the forceps to remove each disc.

4. Check the membranes for damage. Verify that all membrane discs are intact. If membrane damage has occurred, it should be recorded. Data for this sample should be carefully evaluated.
5. using the wooden stirring sticks, scrape the Reagent wells to ensure that all of the precipitate is removed from the bottom of each well. Use one stirring stick fro each sample, starting with lowest volume wells first, the progressing to wells of higher volume. Use a separate stirring stick for each calibrator well. The wells containing Blanking Buffer do not need to be scraped.

G. Transferring 250 µl of Reagent and Blanking Buffer into the 96-well Reading Plate:

1. Set the multichannel pipettor to 260 µl for dispensing.
   Note: 260 µl is used for filling to ensure that no air bubbles are dispensed. The remaining 10 µl must be purged prior to refilling the multichannel pipettor.
2. Transfer 250 µl from each well of the 24-well assay plates to every other well of the 96-well reading plate by columns (see Figure 2, page 7).
3. Change pipette tips and repeat this process for each column.

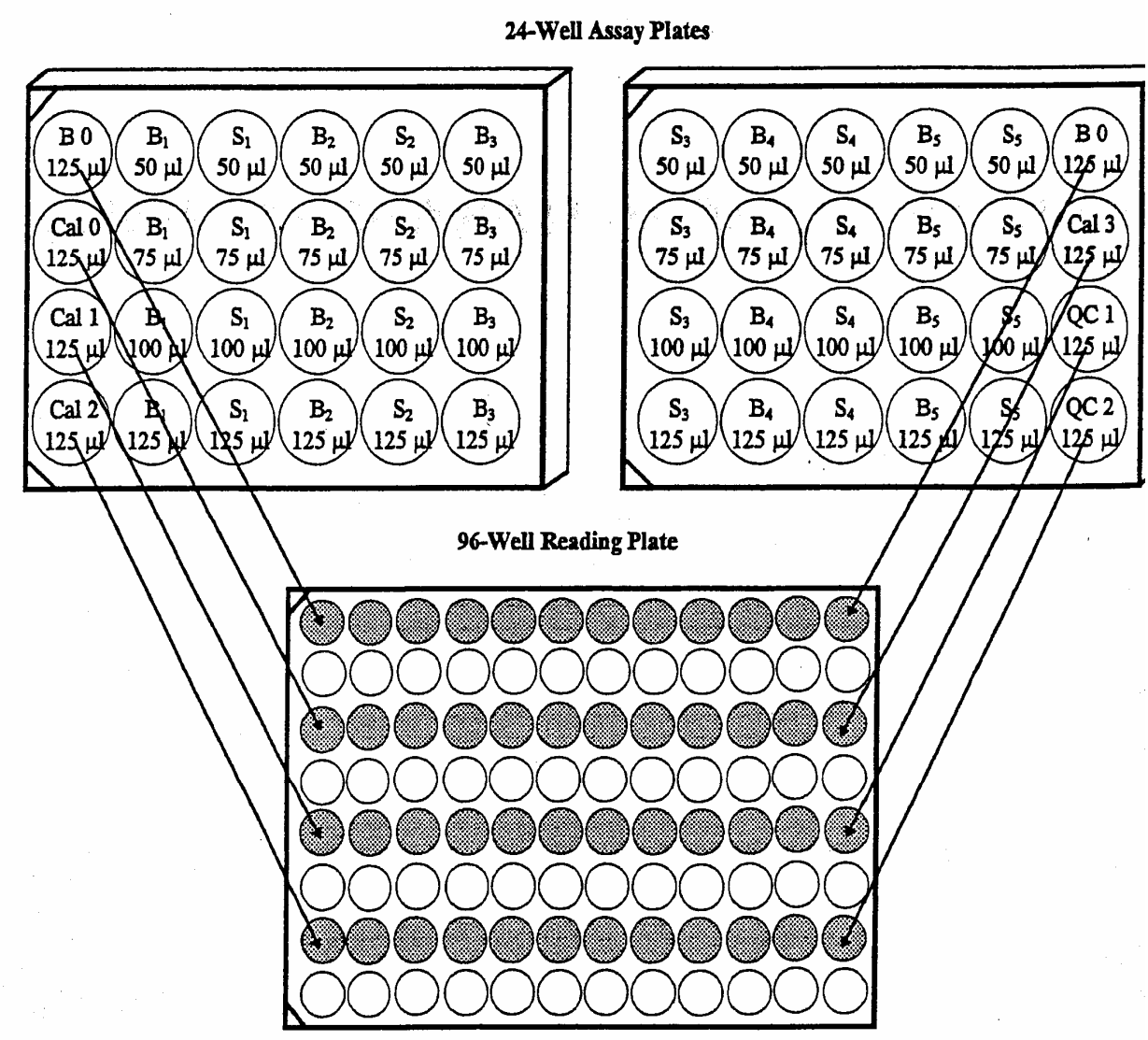


Figure 3.2. Transferring from 24-Well Assay Plates to 96-Well Reading Plate

Figure Legend:

= cell should contain 250 µl of Reagent or Blanking Buffer from the 24-well assay plates

= cell should be empty

H. Reading Assay on Plate Reader (refer to the Irritection Software Instructions found in Chapter 7 for more details:

1. To eliminate inaccurate readings produced by settling of the precipitated Reagent, the assay must be read immediately after transferring the Reagent to the reading plate.
2. If you have the Irritection Software for DOS, follow the instructions listed in Step 2a. If you have the Irritection Software for Windows™, follow the instructions listed in Step 2b.

a. Irritection Software for DOS:

(1) Ensure that the plate set up in the Irritection Software matches the configuration for the assay and that the Calibrator/Quality Control ranges in the software are updated with the current kit lot number.
(2) Select *New Assay Entry* from the *Assay* menu to initiate plate reader.
(3) Remove the reading plate lid, wipe the bottom of the plate with a Kimwipe to remove any moisture and insert the plate into the plate reader with the corner notches facing to the left.
(4) Follow screen prompts to read and analyze data **(read absorbance at 405 nm).**
(5) Upon completion of data entry, press *F10* to process data. If an Inhibition Check needs to be performed, a screen prompt will display the appropriate wells that require inhibition check.

b. Irritection Software for Windows:

(1) From the *Method* menu, choose *Select*.
(2) Select the appropriate assay method.
(3) From the *File* menu, choose *New.*
(4) Select *Assay.*
(5) Select the appropriate protocol.
(6) Select the appropriate plate layout.
(7) A series of screens will be displayed. You will need to enter the appropriate assay, and sample information.
(8) The plate reader will initialize. Remove the reading plate lid, wipe the bottom of the plate with a Kimwipe to remove any moisture and insert the plate into the plate reader with the corner notches facing to the left. Click *Continue,* or press *ENTER.*
(9) After the data collection is completed, a dialog box will be displayed indicating if the assay was qualified or unqualified. Click *OK* to continue.

(10) If an Inhibition Check needs to be performed, a screen prompt will display the appropriate wells that require Inhibition Check.

3. If an Inhibition Check is required, add 25 µl of Inhibition Check to the appropriate wells.
4. Wait five minutes, then re-read.

I. Irritection Software Qualification Checks:

The Irritection software will perform the following qualification checks to ensure assay performance.

1. Calibrator/Quality Control Check: Verifies that Calibrators/Quality Controls are within specified ranges.
2. Net Optical Density Check: Verifies that Net OD ($OD_{reagent} - OD_{blank} = OD_{Net}$) is > -15.
3. Inhibition Check: Verifies that OD of Inhibition Check is greater than OD of Cal 2.
4. Dose Response Check: Verifies that sample dose response curve is either flat or has an increasing curve.

J. Sample Results:

1. Upon acceptance of all qualification checks, the Irritection Software will generate a qualified assay report.
2. Irritection Draize Equivalent (IDE) scores will be calculated and a predicted *in vivo* class will be determined.
3. Customized reports with calibration and dose-response curves may be printed.

K. Clean Up:

1. The Activator, Inhibition Check and all calibrators should be disposed of after the assay has been completed. See MSD Sheets for proper disposal procedures.

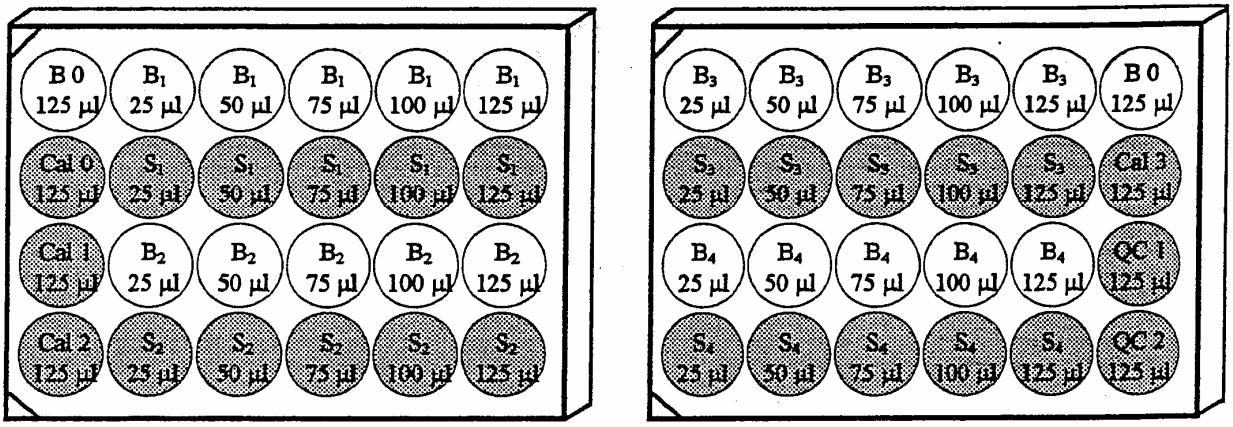


Figure 3.3. Plate Configuration – Alternative I
Suitable Analysis for Four Samples at Five Volumes

Figure Legend:

= well contains Blanking Buffer

= well contains Reagent

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 25, 50, 75, 100 and 125 µl/mg of sample applied to membrane discs.
S: 1.25 ml Activated Reagent in well, 25, 50, 75, 100 and 125 µl/mg of sample applied to membrane discs.

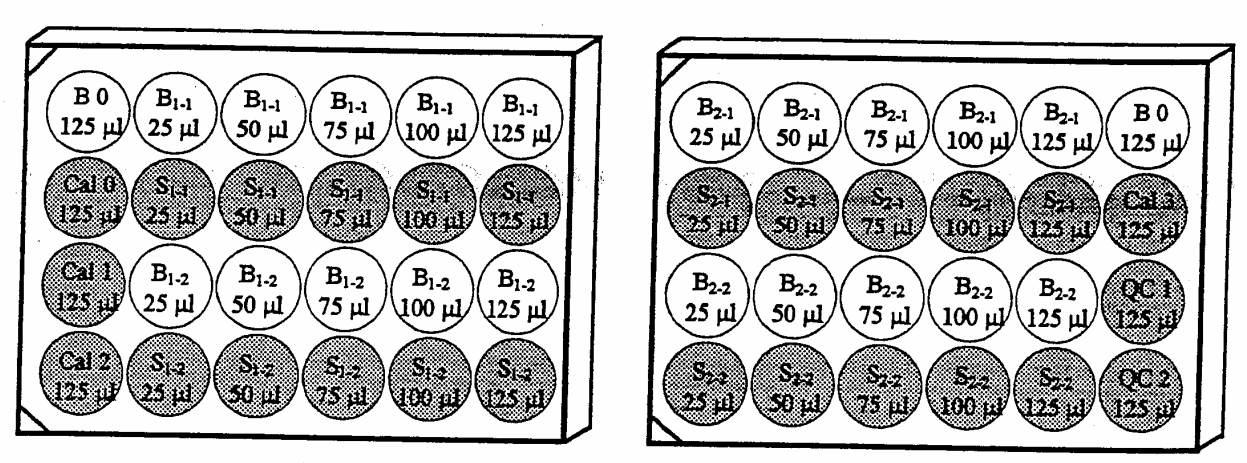


Figure 3.4. Plate Configuration – Alternative II
Suitable Analysis for Two Samples at Five Volumes in Duplicates

Figure Legend:

= well contains Blanking Buffer = well contains Reagent

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 25, 50, 75, 100 and 125 µl/mg of sample applied to membrane discs.
S: 1.25 ml Activated Reagent in well, 25, 50, 75, 100 and 125 µl/mg of sample applied to membrane discs.

DERMAL RESPONSE ASSAY
VOLUME-DEPENDENT DOSE-RESPONSE PROTOCOL
(HUMAN IRRITANCY EQUIVALENT SCORING SYSTEM)

In Vitro International's Dermal Response Assay is an *in vitro* assay method that may be employed to determine and predict the dermal irritation potential of non-surfactant based chemical, petrochemical, and cosmetic samples. The results of this test have been shown to correlate with those obtained with the *in vivo* human patch test. Dermal Response findings may be related to the *in vivo* test results through the Human Irritancy Equivalent (HIE) scoring system that has been developed for this purpose.

This assay protocol provides a detailed description of the experimental procedures that are required to perform the Dermal Response Assay. The protocol consists of five steps:

1. Preparation of the samples
2. Preparation of the dermal protein reagent solution
3. Set-up of the assay
4. Incubation for 24 hours to permit reaction of the samples and protein reagent.
5. Determination and interpretation of the assay results.

The first three steps may be completed in approximately two hours. The final step, to be performed on the following day, may be completed in approximately one hour.

Prior to beginning the assay, place the Hydrating Solution in a 25˚C incubator for 1 to 2 hours to ensure that it is approximately 25˚C. Remove the 24-well assay plates filled with membrane discs from the refrigerator. Allow the membrane discs to equilibrate to room temperature (approximately 30-45 minutes) before being used in Step III.D.3.

I. Instrumentation, Reagents and Materials

A. Kit Contains:
   15. Dermal Reagent Powder (1 Bottle)
   16. Dermal Hydrating solution (1 Bottle)
   17. Dermal Blanking Buffer (1 Bottle)
   18. Dermal Inhibition Check (1 Vial)
   19. Dermal Activator (1 Vial)
   20. Four Calibrator Solutions: Cal 0, Cal 1, Cal 2, Cal 3
   21. Two Quality Control Solutions: QC 1, QC 2
   22. 96-well Reading Plate (1)

23. 24-well Assay Plates Filled with Membrane Discs (26 pink discs and 22 clear discs)–**store at 2-8˚C (2)**
24. Wooden Stirring Sticks (12)
25. Whatman #1 Filter Paper, 12.5 cm diameter (1)
26. Procedural Summary
27. Range Specification Data Sheet
28. Irritection Software Instructions

B. Additional Materials Required:

29. Plate Reader (such as Modified Cambridge 7520 Microplatereader)
30. IBM compatible PC with Irritection Software
31. Incubator Maintained at 25˚C ± 1˚C
32. Balance (110 g Capacity)
33. pH Meter
34. Vortex Mixer
35. Positive Displacement Micro Pipettor (such as Labindustries Popette™ Micropipettor Model P-250, 0.5 – 250 µl)
36. Multichannel Pipettor (such as Matrix 6 Channel EXP Impact Pipettor 15-1250 µl)
37. Disposable Reservoir Trays (2—such as 100 ml Matrix Disposable Reagent Reservoir)
38. 100 ml Graduated Cylinder
39. 100 ml Beaker
40. Funnel (such as Oxford Vented Disposable Polystyrene Funnel, 12.5 cm diameter)
41. Plastic Forceps
42. Plastic Wrap

II. Sample Preparation

B. Sample Preparation

Typically, five samples may be analyzed at four volumes (50, 75, 100, 125 µl).

1. If the sample is a liquid, dilute the sample to the desired concentration for analysis.
2. If the sample is a solid, attempt to convert in to a liquid by gently warming it. If the sample melts with warming, apply the liquid molten following procedures detailed in Step III.D.3.
3. If the sample is a solid that does not readily melt with warming, weigh out 50, 75, 100, and 125 mg of each sample and place each amount directly into the membrane discs. (If you choose to evaluate 4 samples at 5 volumes, 25 mg should be used as the fifth weight for analysis.) This should be completed prior to rehydrating the Reagent as described in Section III.A below.

III. Procedures

A. Dermal Reagent Preparation:

1. Rehydration:
    a. Remove the Hydrating Solution from the 25˚C incubator.
    b. Using a funnel, pour the entire contects of the Hydrating Solution into the Reagent Powder bottle.
    c. Gently swirl until all of the Reagent Powder is dissolved.
    d. Let the dissolved Reagent stand at room temperature for approximately 10 minutes before filtering.

2. Filtration:
    a. Fold the filter paper and place in funnel.
    b. Pour all of the dissolved Reagent into the funnel and collect the filtrate in a graduated cylinder at atmospheric pressure (do not use a vacuum pump.). This will take approximately 10 minutes.
    Note: During the filtration process, label the 24-well assay plates (Step III.B).
    c. Pour 40 ml of the filtered Reagent into a 100 ml beaker.
    Note: The Reagent must be used within 30 minutes of rehydration.

3. Activation:
    a. Record the initial pH and temperature of the filtered Reagent, verifying that it falls within the following specified ranges: **pH: 9.79-10.49 and T: 23-25˚C.**
    b. Using the positive displacement pipettor, slowly add 920 µl of the Activator to the filtered Reagent. Gently swirl to mix.
    c. Record the final pH of the Activated Reagent solution, verifying that it falls within the following specified range: **pH: 7.48-8.36.** Note: If the pH does not fall within the specified range, recalibrate the pH meter, gently swirl the Reagent and remeasure the pH. If the pH is still not within the specified range, contact your technical representative at 1-800-2-INVITRO.
    d. Using the positive displacement pipettor, slowly add 690 µl of the Activator to the bottle of Blanking Buffer. Gently swirl to mix.
    e. It is not necessary to measure the pH or temperature of the Activated Blanking Buffer.

B. Labeling the 24-Well Assay Plates:

1. Remove the membrane discs from the assay plates and place them in a container. These discs will be used in Step D.1
2. Align the assay plates with the notched corners toward the left and label their lids. A typical plate configuration that has been employed to evaluate five different samples at four different volumes is shows in Figure 3.9 on the next page.

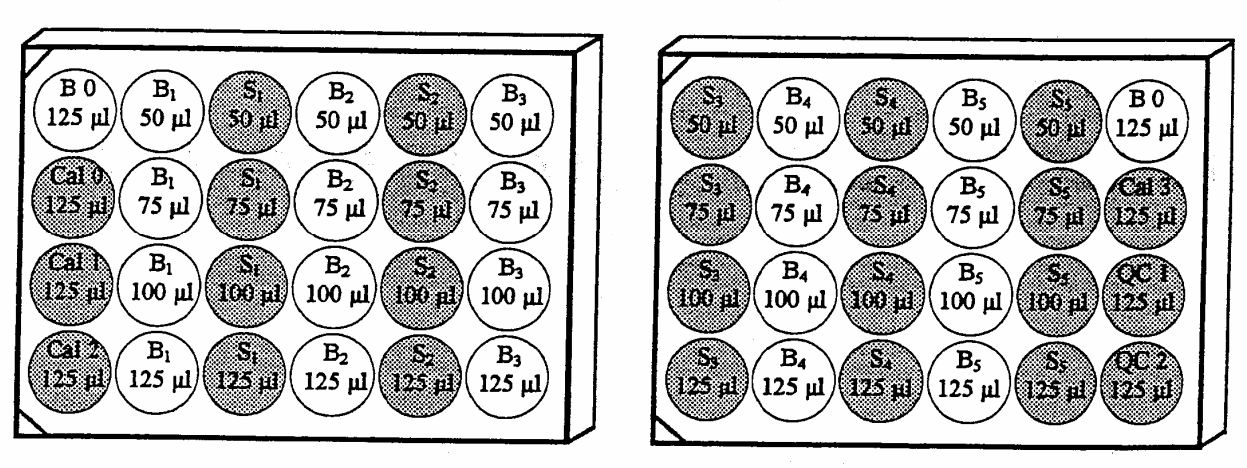


Figure 3.5. Suggested Plate Configuration: Analysis of Five Samples at Four Volumes

Figure Legend:

= well contains Blanking Buffer and an clear disc

= well contains Reagent and a pink disc

B 0:1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to clear membrane disc.
Cal 0:1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to pink membrane disc.
Cal 1:1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to pink membrane disc.
Cal 2:1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to pink membrane disc.
Cal 3:1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to pink membrane disc.
QC 1:1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to pink membrane disc.
QC 2:1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to pink membrane disc.
B:1.25 ml Activated Blanking Buffer in well, 50, 75, 100 and 125 µl/mg of sample applied to clear membrane discs.
S:1.25 ml Activated Reagent in well, 50, 75, 100 and 125 µl/mg of sample applied to pink membrane discs.

Note: Alternative assay schemes are shown in Figure 3.7 and 3.8 on pages 26 and 27 of the this chapter.

C. Addition of Reagent and Blanking Buffer to 24-Well Assay Plates:

1. Remove the labeled lid on each assay plate and use the lid or Figure 1 as a reference when filling the wells.
2. Set the multichannel pipettor at 1250 µl.

3. Pour the Activated Reagent into a disposable reservoir tray.
4. Using the multichannel pipettor, add 1250 µl of the Activated Reagent to the following wells: Cal 0, Cal 1, Cal 2, Cal 3, QC 1, QC 2 and all wells designated for analysis of the samples (S).
5. Pour the Activated Blanking Buffer into a disposable reservoir tray.
6. Change pipette tips and add 1250 µl of the Blanking Buffer to the wells designated B 0 on both plates and all other wells designated as blanks (B).

D. Addition of Calibrators, Quality Controls and Samples:

Note: Avoid cross contamination among the separate Calibrator, Quality Control and sample wells by utilizing a clean pipette tip for each.

1. Pipette 125 µl of Cal 0 into a clear membrane disc and insert it into the B 0 well. Repeat this procedure for the B 0 well of the second assay plate,
2. Pipette 125 µl of Cal 0, Cal 1, Cal 2, Cal 3, QC 1 and QC 2 into pink membrane discs and insert them into the appropriate wells of the assay plates.
3. If the sample is a liquid, pipette 50, 75, 100 and 125 µl of each test sample into the clear and pink membrane discs and insert them into the corresponding blank and test sample wells of the assay plates. (If you wish to evaluate 4 samples at 5 volumes, 25 µl should be used as the fifth volume for analysis.)
4. If the sample is a solid, insert the membrane discs which contain 50, 75, 100 and 125 mg of each test sample into the corresponding blank and test sample wells of the plates.

E. Incubation:

1. To prevent evaporation of the samples from the membrane discs, tightly wrap the assay plate with plastic wrap. Place a lid on top of each wrapped plate.
2. Record the technician's name, date and time on the lids of the assay plates.
3. Place the assay plates in an incubator maintained at 25˚C ± 1˚C for 24 hours (± 30 minutes).

F. Removing Membrane Discs:

1. Remove the assay plates from the incubator.
2. Remove the lids from the assay plates and save them. They will be used as a reference when transferring the Reagent and the Blanking Buffer to the clean 96-well reading plate.
3. Remove the plastic wrap from each assay plate.
4. Remove each membrane disc individually with plastic forceps.

5. Check the membranes for damage. Verify that all membrane discs are intact. If membrane damage has occurred, it should be recorded. Data for this sample should be carefully evaluated.
6. Using the wooden stirring sticks, scrape the Reagent wells to ensure that all of the precipitate is removed from the bottom of each well. Use one stirring stick for each sample, starting with lowest volume wells first, the progressing to wells of higher volume. Use a separate stirring stick for each calibrator well. The wells containing Blanking Buffer do not need to be scraped.
7. Note any wells with reduced volume. Reduced cell well volumes may be indicative of hygroscopic effects or technical problems and should be recorded.

G. Transferring 250 µl of Reagent and Blanking Buffer into the 96-well Reading Plate:

1. Set the multichannel pipettor to 260 µl for dispensing.
   Note: 260 µl is used for filling to ensure that no air bubbles are dispensed. The remaining 10 µl must be purged prior to refilling the multichannel pipettor.
2. Transfer 250 µl from each well of the 24-well assay plates to every other well of the 96-well reading plate by columns (see Figure 2, page 7).
3. Change pipette tips and repeat this process for each column.

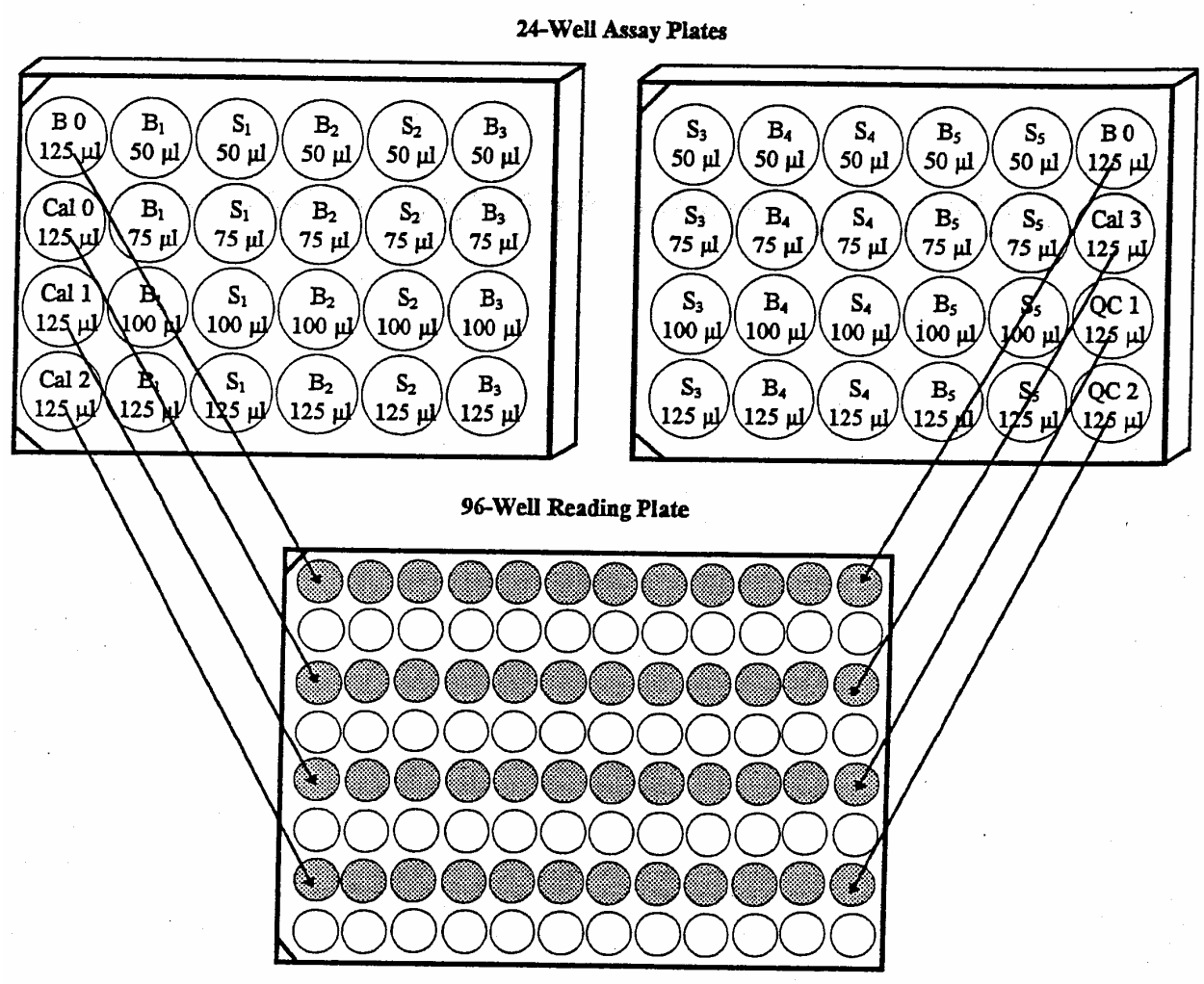


Figure 3.6. Transferring from 24-Well Assay Plates to 96-Well Reading Plate

Figure Legend:

= cell should contain 250 µl of Reagent or Blanking Buffer from the 24-well assay plates

= cell should be empty

H. Reading Assay on Plate Reader (refer to the Irritection Software Instructions found in Chapter 7 for more details:

1. To eliminate inaccurate readings produced by settling of the precipitated Reagent, the assay must be read immediately after transferring the Reagent to the reading plate.
2. If you have the Irritection Software for DOS, follow the instructions listed in Step 2a. If you have the Irritection Software for Windows™, follow the instructions listed in Step 2b.

a. Irritection Software for DOS:

(1) Ensure that the plate set up in the Irritection Software matches the configuration for the assay and that the Calibrator/Quality Control ranges in the software are updated with the current kit lot number.
(2) Select *New Assay Entry* from the *Assay* menu to initiate plate reader.
(3) Remove the reading plate lid, wipe the bottom of the plate with a Kimwipe to remove any moisture and insert the plate into the plate reader with the corner notches facing to the left.
(4) Follow screen prompts to read and analyze data **(read absorbance at 450 nm).**
(5) Upon completion of data entry, press *F10* to process data. If an Inhibition Check needs to be performed, a screen prompt will display the appropriate wells that require inhibition check.

b. Irritection Software for Windows:

(1) From the *Method* menu, choose *Select*.
(2) Select the appropriate assay method.
(3) From the *File* menu, choose *New.*
(4) Select *Assay.*
(5) Select the appropriate protocol.
(6) Select the appropriate plate layout.
(7) A series of screens will be displayed. You will need to enter the appropriate assay, and sample information.
(8) The plate reader will initialize. Remove the reading plate lid, wipe the bottom of the plate with a Kimwipe to remove any moisture and insert the plate into the plate reader with the corner notches facing to the left. Click *Continue,* or press *ENTER.*
(9) After the data collection is completed, a dialog box will be displayed indicating if the assay was qualified or unqualified. Click *OK* to continue.

(10) If an Inhibition Check needs to be performed, a screen prompt will display the appropriate wells that require Inhibition Check.

3. If an Inhibition Check is required, add 25 µl of Inhibition Check to the appropriate wells.
4. Wait five minutes, then re-read.

I. Irritection Software Qualification Checks:

The Irritection software will perform the following qualification checks to ensure assay performance.

1. Calibrator/Quality Control Check: Verifies that Calibrators/Quality Controls are within specified ranges.
2. Net Optical Density Check: Verifies that Net OD ($OD_{reagent} - OD_{blank} = OD_{Net}$) is > -15.
3. Inhibition Check: Verifies that OD of Inhibition Check is greater than OD of Cal 2.
4. Dose Response Check: Verifies that sample dose response curve is an increasing or flat curve.

J. Sample Results:

1. Upon acceptance of all qualification checks, the Irritection Software will generate a qualified assay report.
2. Human Irritancy Equivalent (HIE) scores will be calculated and a predicted *in vivo* class will be determined.
3. Customized reports with calibration and dose-response curves may be printed.

K. Clean Up:

1. The Activator, Inhibition Check and all calibrators should be disposed of after the assay has been completed. See MSD Sheets for proper disposal procedures.

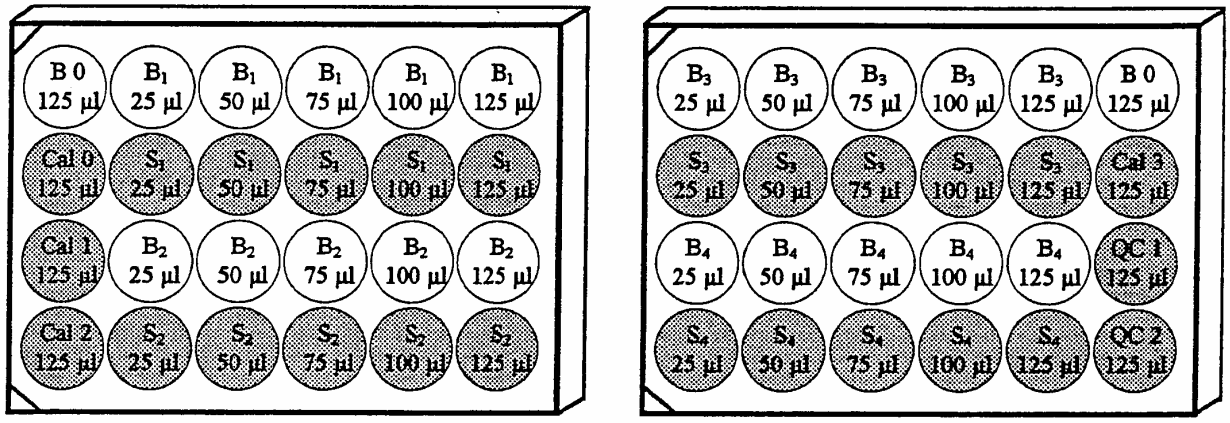


Figure 3.7. Plate Configuration – Alternative I
Suitable for Four Samples at Five Volumes

Figure Legend:

= well contains Blanking Buffer and a clear disc

= well contains Reagent and a pink disc

B 0:1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to clear membrane disc.
Cal 0:1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to pink membrane disc.
Cal 1:1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to pink membrane disc.
Cal 2:1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to pink membrane disc.
Cal 3:1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to pink membrane disc.
QC 1:1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to pink membrane disc.
QC 2:1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to pink membrane disc.
B:1.25 ml Activated Blanking Buffer in well, 25, 50, 75, 100 and 125 µl/mg of sample applied to clear membrane discs.
S: 1.25 ml Activated Reagent in well, 25, 50, 75, 100 and 125 µl/mg of sample applied to pink membrane discs.

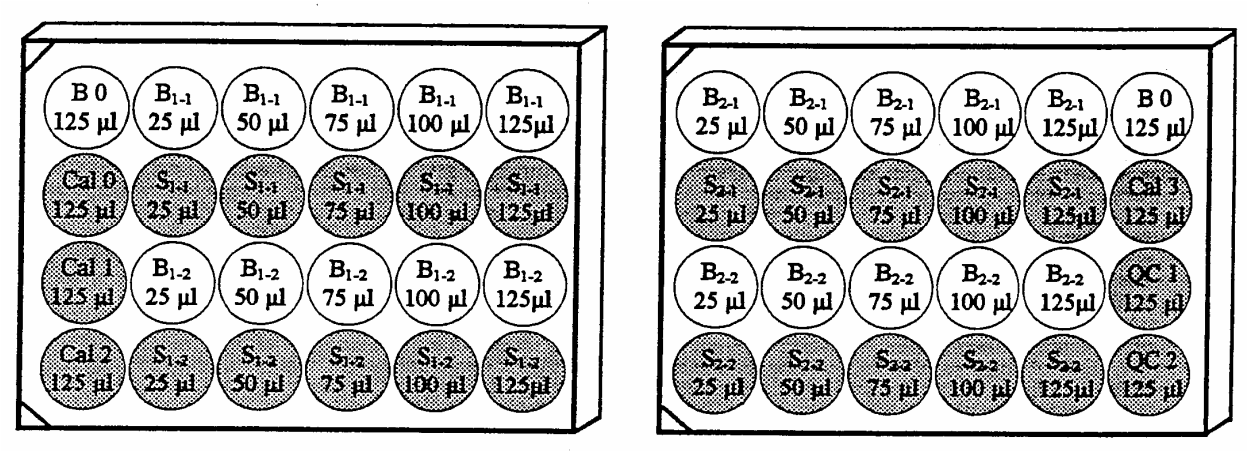


Figure 3.8. Plate Configuration – Alternative II
Suitable for Two Samples at Five Volumes in Duplicates

Figure Legend:

= well contains Blanking Buffer and a clear disc

= well contains Reagent and a pink disc

B 0:1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to clear membrane disc.
Cal 0:1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to pink membrane disc.
Cal 1:1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to pink membrane disc.
Cal 2:1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to pink membrane disc.
Cal 3:1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to pink membrane disc.
QC 1:1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to pink membrane disc.
QC 2:1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to pink membrane disc.
B:1.25 ml Activated Blanking Buffer in well, 25, 50, 75, 100 and 125 µl/mg of sample applied to clear membrane discs.
S:1.25 ml Activated Reagent in well, 25, 50, 75, 100 and 125 µl/mg of sample applied to pink membrane discs.

OCULAR RESPONSE ASSAY
CONCENTRATION-DEPENDENT DOSE-RESPONSE PROTOCOL
(DRAIZE EQUIVALENT SCORING SYSTEM)

In Vitro International's Ocular Response Assay is an *in vitro* assay method that may be employed to determine and predict the ocular irritation potential of surfactant and surfactant - based samples. The results of this test have been shown to correlate with those obtained with the *in vivo* Draize test. Ocular Response findings may be related to the *in vivo* test results through the Irritection Draize Equivalent (IDE) scoring system that has been developed for this purpose.

This assay protocol provides a detailed description of the experimental procedures that are required to perform the Ocular Response Assay. The protocol consists of five steps:

1. Preparation of the samples
2. Preparation of the Ocular protein reagent solution
3. Set-up of the assay
4. Incubation for 24 hours to permit reaction of the samples and protein reagent.
5. Determination and interpretation of the assay results.

The first three steps may be completed in approximately two hours. The final step, to be performed on the same day, may be completed in approximately one hour.

Prior to beginning the assay, place the Hydrating Solution in a 25˚C incubator for 1 to 2 hours to ensure that it is approximately 25˚C. Remove the 24-well assay plates filled with membrane discs from the refrigerator. Allow the membrane discs to equilibrate to room temperature (approximately 30-45 minutes) before being used in Step III.D.3.

I. Instrumentation, Reagents and Materials

A. Kit Contains:
   1. Ocular Reagent Powder (1 Bottle)
   2. Ocular Hydrating solution (1 Bottle)
   3. Ocular Blanking Buffer (1 Bottle)
   4. Ocular Inhibition Check (1 Vial)
   5. Ocular Activator (1 Vial)
   6. Four Calibrator Solutions: Cal 0, Cal 1, Cal 2, Cal 3
   7. Two Quality Control Solutions: QC 1, QC 2
   8. 24-well Assay Plates Filled with Membrane Discs (48 clear discs)–**store at 2-8˚C (2)**

9. 96-well Reading Plate (1)
10. Wooden Stirring Sticks (12)
11. Whatman #1 Filter Paper, 12.5 cm diameter (1)
12. Procedural Summary
13. Range Specification Data Sheet
14. Irritection Software Instructions

B. Additional Materials Required:
1. Plate Reader (such as Modified Cambridge 7520 Microplatereader)
2. IBM compatible PC with Irritection Software
3. Incubator Maintained at 25˚C ± 1˚C
4. Balance (110 g Capacity)
5. pH Meter
6. Vortex Mixer
7. Positive Displacement Micro Pipettor (such as Labindustries Popette™ Micropipettor Model P-250, 5 – 250 µl)
8. Multichannel Pipettor (such as Matrix 6 Channel EXP Impact Pipettor 15-1250 µl)
9. Disposable Reservoir Trays (2—such as 100 ml Matrix Disposable Reagent Reservoir)
10. 100 ml Graduated Cylinder
11. 100 ml Beaker
12. Funnel (such as Oxford Vented Disposable Polystyrene Funnel, 12.5 cm diameter)
13. Plastic Forceps
14. Plastic Wrap

II. Sample Preparation

A. Concentration-Dependent Dose-Response Study: Solids

Five solid samples may be evaluated at four different concentrations. Obtain fine shavings from different locations on a solid material, e.g., a bar of soap.

1. Weigh out the required amount of sample and place it in a beaker of suitable size.
2. Add the appropriate amount of distilled water to the beaker.
3. Mix on a magnetic stir plate or use a vortex mixer to achieve dissolution of the solid.

A typical range of concentrations could be prepared according to the following scheme:

Concentration (%)	Sample Weight (g)	Volume Water (ml)
15	1.50	10.0
8	0.80	10.0
5	0.50	10.0
2	0.20	10.0
1	0.10	10.0

B. Concentration-Dependent Dose-Response Study: Liquids

Five liquid samples may be evaluated at four different concentrations.

1. Dilute liquid samples to the desired concentrations using distilled water as the diluent.

A typical range of concentrations could be prepared according to the following scheme:

Concentration (%)	Volume Neat Sample (ml)	Volume Water (ml)
50	0.50	0.50
25	0.25	0.75
10	0.10	0.90
5	0.05	0.95
1	0.01	0.99

Note: Sample preparation should be completed prior to proceeding to Step III.

III. Procedures

A. Dermal Reagent Preparation:

1. Rehydration:
    a. Remove the Hydrating Solution from the 25˚C incubator.
    b. Using a funnel, pour the entire contents of the Hydrating Solution into the Reagent Powder bottle.
    c. Gently swirl until all of the Reagent Powder is dissolved.
    d. Let the dissolved Reagent stand at room temperature for approximately 10 minutes before filtering.

2. Filtration:
    a. Fold the filter paper and place in funnel.
    b. Pour all of the dissolved Reagent into the funnel and collect the filtrate in a graduated cylinder at atmospheric pressure (do not use a vacuum pump.). This will take approximately 10 minutes.

Note: During the filtration process, label the 24-well assay plates (Step III.B).

c. Pour 40 ml of the filtered Reagent into a 100 ml beaker.
Note: The Reagent must be used within 30 minutes of rehydration.

3. Activation:
   a. Record the initial pH and temperature of the filtered Reagent, verifying that it falls within the following specified ranges: **pH: 7.91-8.19 and T: 23-25˚C.**
   b. Using the positive displacement pipettor, slowly add 2400 µl of the Activator to the filtered Reagent. Gently swirl to mix.
   c. Record the final pH of the Activated Reagent solution, verifying that it falls within the following specified range: **pH: 5.80-5.98.** Note: If the pH does not fall within the specified range, recalibrate the pH meter, gently swirl the Reagent and remeasure the pH. If the pH is still not within the specified range, contact your technical representative at 1-800-2-INVITRO.
   d. Using the positive displacement pipettor, slowly add 1800 µl of the Activator to the bottle of Blanking Buffer. Gently swirl to mix.
   e. It is not necessary to measure the pH or temperature of the Activated Blanking Buffer.

B. Labeling the 24-Well Assay Plates:

1. Remove the membrane discs from the assay plates and place them in a container. These discs will be used in Step D.1
2. Align the assay plates with the notched corners toward the left and label their lids. A typical plate configuration that has been employed to evaluate five different samples at four different volumes is shows in Figure 3.5.

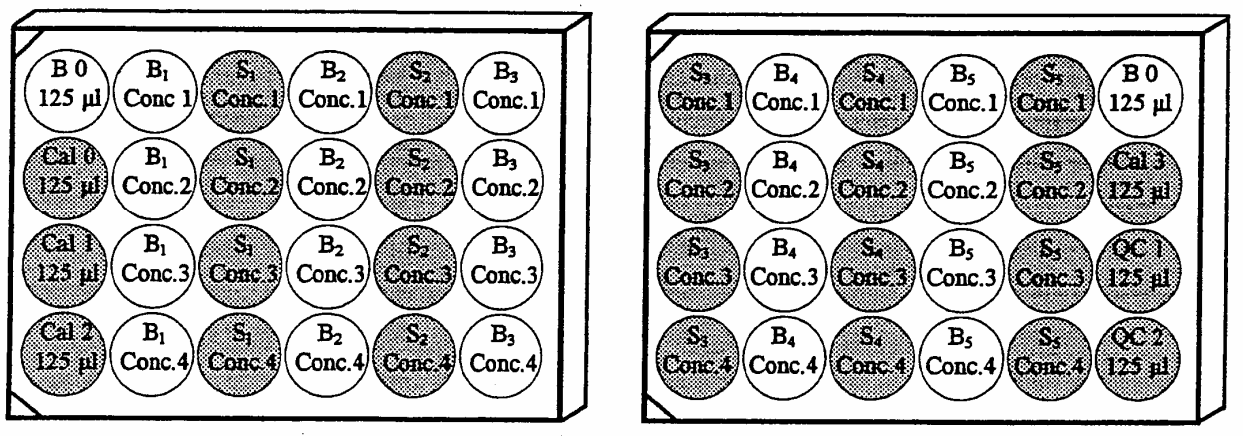


Figure 3.9. Suggested Plate Configuration: Analysis of Five Samples at Four Different Concentrations

Figure Legend:

= well contains Blanking Buffer = well contains Reagent

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 125 µl of sample solution at concentrations 1, 2, 3 and 4 applied to membrane discs.
S: 1.25 ml Activated Reagent in well, 125 µl of sample solution at concentrations 1, 2, 3 and 4 applied to membrane discs.

Note: Alternative assay schemes are shown in Figure 3.11 and 3.12 on pages 38 and 39 of this chapter.

C. Addition of Reagent and Blanking Buffer to 24-Well Assay Plates:

1. Remove the labeled lid on each assay plate and use the lid or Figure 1 as a reference when filling the wells.

2. Set the multichannel pipettor at 1250 µl.
3. Pour the Activated Reagent into a disposable reservoir tray.
4. Using the multichannel pipettor, add 1250 µl of the Activated Reagent to the following wells: Cal 0, Cal 1, Cal 2, Cal 3, QC 1, QC 2 and all wells designated for analysis of the samples (S).
5. Pour the Activated Blanking Buffer into a disposable reservoir tray.
6. Change pipette tips and add 1250 µl of the Blanking Buffer to the wells designated B 0 on both plates and all other wells designated as blanks (B).

D. Addition of Calibrators, Quality Controls and Samples:

Note: Avoid cross contamination among the separate Calibrator, Quality Control and sample wells by utilizing a clean pipette tip for each.

1. Pipette 125 µl of Cal 0 into a clear membrane disc and insert it into the B 0 well. Repeat this procedure for the B 0 well of the second assay plate,
2. Pipette 125 µl of Cal 0, Cal 1, Cal 2, Cal 3, QC 1 and QC 2 into the membrane discs and insert them into the appropriate wells of the assay plates.
3. Pipette 125 µl of each test sample concentration into the membrane discs and insert them into the corresponding blank and test sample wells of the assay plates.

E. Incubation:

1. Tightly wrap the assay plate with plastic wrap. Place a lid on top of each wrapped plate.
2. Record the technician's name, date and time on the lids of the assay plates.
3. Place the assay plates in an incubator maintained at 25˚C ± 1˚C for 5 hours (± 10 minutes).

F. Removing Membrane Discs:

1. Remove the assay plates from the incubator.
2. Remove the lids from the assay plates and save them. They will be used as a reference when transferring the Reagent and the Blanking Buffer to the clean 96-well reading plate.
3. Carefully remove the plastic wrap from each assay plate and use the forceps to remove each disc.
4. Check the membranes for damage. Verify that all membrane discs are intact. If membrane damage has occurred, it should be recorded. Data for this sample should be carefully evaluated.
5. Using the wooden stirring sticks, scrape the Reagent wells to ensure that all of the precipitate is removed from the bottom of each well. Use one stirring stick for

each sample, starting with lowest volume wells first, the progressing to wells of higher volume. Use a separate stirring stick for each calibrator well. The wells containing Blanking Buffer do not need to be scraped.

G. Transferring 250 µl of Reagent and Blanking Buffer into the 96-well Reading Plate:

1. Set the multichannel pipettor to 260 µl for filling and 250 µl for dispensing.
   Note: 260 µl is used for filling to ensure that no air bubbles are dispensed. The remaining 10 µl must be purged prior to refilling the multichannel pipettor.
2. Transfer 250 µl from each well of the 24-well assay plates to every other well of the 96-well reading plate by columns (see Figure 2, page 8).
3. Change pipette tips and repeat this process for each column.

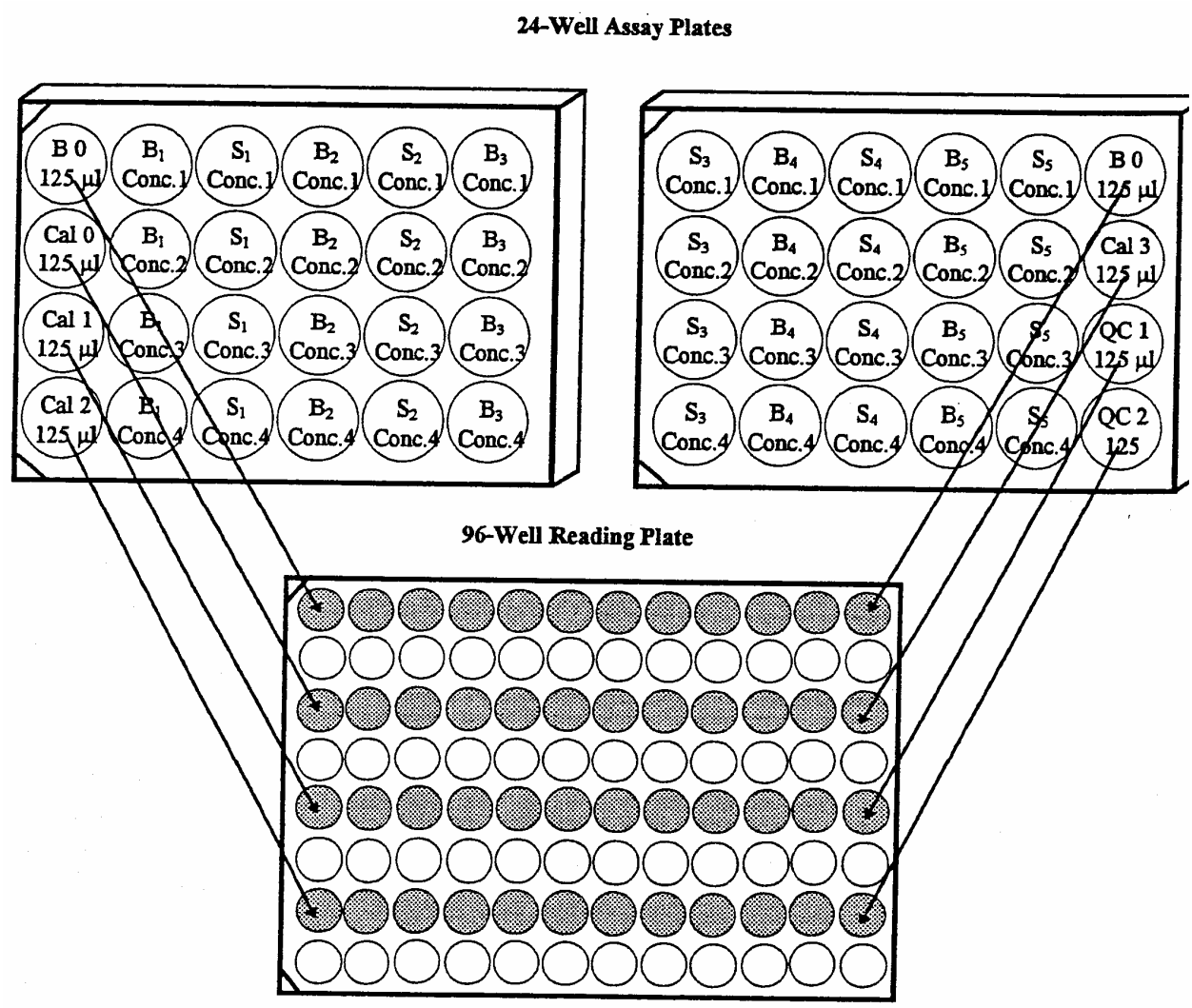


Figure 3.10. Transferring from 24-Well Assay Plates to 96-Well Reading Plate

Figure Legend:

= cell should contain 250 µl of Reagent or Blanking Buffer from the 24-well assay plates

= cell should be empty

H. Reading Assay on Plate Reader (refer to the Irritection Software Instructions found in Chapter 7 for more details:

1. To eliminate inaccurate readings produced by settling of the precipitated Reagent, the assay must be read immediately after transferring the Reagent to the reading plate.
2. If you have the Irritection Software for DOS, follow the instructions listed in Step 2a. If you have the Irritection Software for Windows™, follow the instructions listed in Step 2b.

a. Irritection Software for DOS:

(1) Ensure that the plate set up in the Irritection Software matches the configuration for the assay and that the Calibrator/Quality Control ranges in the software are updated with the current kit lot number.
(2) Select *New Assay Entry* from the *Assay* menu to initiate plate reader.
(3) Remove the reading plate lid, wipe the bottom of the plate with a Kimwipe to remove any moisture and insert the plate into the plate reader with the corner notches facing to the left.
(4) Follow screen prompts to read and analyze data **(read absorbance at 405 nm).**
(5) Upon completion of data entry, press *F10* to process data. If an Inhibition Check needs to be performed, a screen prompt will display the appropriate wells that require inhibition check.

b. Irritection Software for Windows:

(1) From the *Method* menu, choose *Select*.
(2) Select the appropriate assay method.
(3) From the *File* menu, choose *New.*
(4) Select *Assay.*
(5) Select the appropriate protocol.
(6) Select the appropriate plate layout.
(7) A series of screens will be displayed. You will need to enter the appropriate assay, and sample information.
(8) The plate reader will initialize. Remove the reading plate lid, wipe the bottom of the plate with a Kimwipe to remove any moisture and insert the plate into the plate reader with the corner notches facing to the left. Click *Continue,* or press *ENTER.*
(9) After the data collection is completed, a dialog box will be displayed indicating if the assay was qualified or unqualified. Click *OK* to continue.

(10) If an Inhibition Check needs to be performed, a screen prompt will display the appropriate wells that require Inhibition Check.

3. If an Inhibition Check is required, add 25 µl of Inhibition Check to the appropriate wells.
4. Wait five minutes, then re-read.

I. Irritection Software Qualification Checks:

The Irritection software will perform the following qualification checks to ensure assay performance.

1. Calibrator/Quality Control Check: Verifies that Calibrators/Quality Controls are within specified ranges.
2. Net Optical Density Check: Verifies that Net OD ($OD_{reagent} - OD_{blank} = OD_{Net}$) is > -15.
3. Inhibition Check: Verifies that OD of Inhibition Check is greater than OD of Cal 2.
4. Concentration Curve Check: Verifies that sample concentration curve is a parabolic curve.
5. Dose Response Check: Verifies that sample dose response curve is an increasing or flat curve.

J. Sample Results:

1. Upon acceptance of all qualification checks, the Irritection Software will generate a qualified assay report.
2. Irritection Draize Equivalent (IDE) scores will be calculated and a predicted *in vivo* class will be determined.
3. Customized reports with calibration and dose-response curves may be printed.

K. Clean Up:

1. The Activator, Inhibition Check and all calibrators should be disposed of after the assay has been completed. See MSD Sheets for proper disposal procedures.

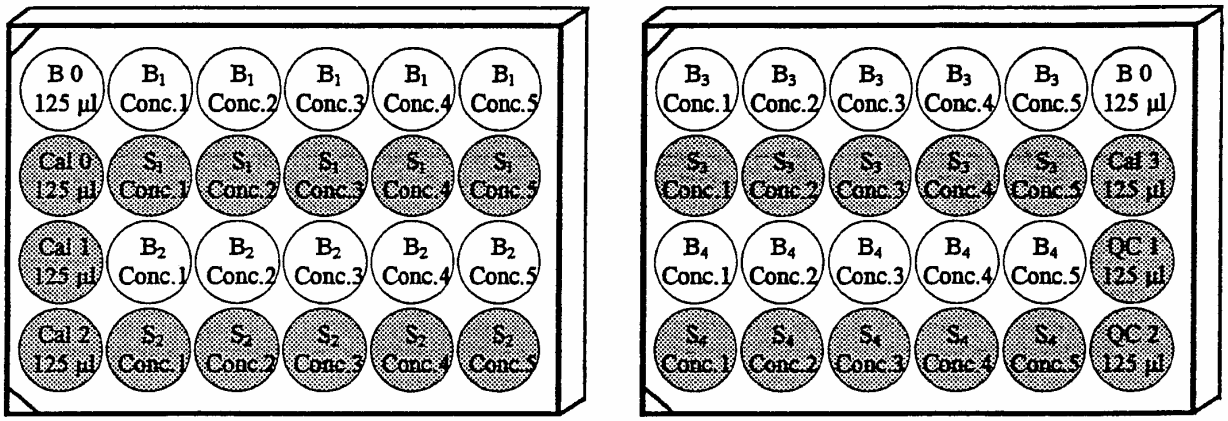


Figure 3.11. Plate Configuration – Alternative I
Suitable for Four Samples at Five Concentrations

Figure Legend:

= well contains Blanking Buffer = well contains Reagent

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 125 µl of sample at concentrations 1, 2, 3, 4 and 5 applied to membrane discs.
S: 1.25 ml Activated Reagent in well, 125 µl of sample at concentrations 1, 2, 3, 4 and 5 applied to membrane discs.

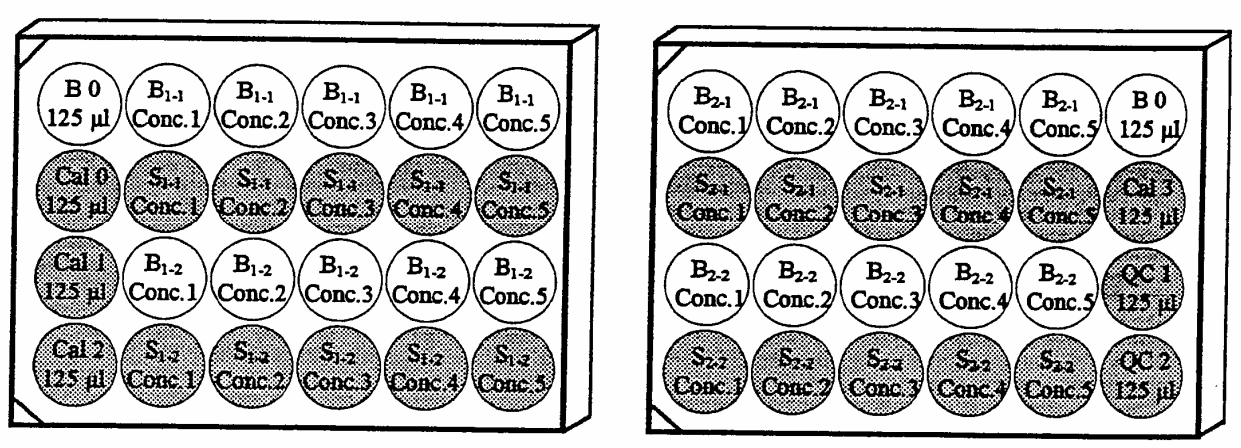


Figure 3.12. Plate Configuration – Alternative II
Suitable for Two Samples at Five Concentrations in Duplicates

Figure Legend:

= well contains Blanking Buffer

= well contains Reagent

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 25 µl/mg of sample at concentrations 1, 2, 3, 4 and 5 applied to membrane discs.
S: 1.25 ml Activated Reagent in well, 125 µl of sample at concentrations 1, 2, 3, 4 and 5 applied to membrane discs.

DERMAL RESPONSE ASSAY
CONCENTRATION-DEPENDENT DOSE-RESPONSE PROTOCOL
(HUMAN IRRITANCY EQUIVALENT SCORING SYSTEM)

In Vitro International's Dermal Response Assay is an *in vitro* assay method that may be employed to determine and predict the dermal irritation potential of surfactant and surfactant - based samples. The results of this test have been shown to correlate with those obtained with the *in vivo* human patch test. Dermal Response findings may be related to the *in vivo* test results through the Human Irritancy Equivalent (HIE) scoring system that has been developed for this purpose.

This assay protocol provides a detailed description of the experimental procedures that are required to perform the Dermal Response Assay. The protocol consists of five steps:

1. Preparation of the samples
2. Preparation of the dermal protein reagent solution
3. Set-up of the assay
4. Incubation for 24 hours to permit reaction of the samples and protein reagent.
5. Determination and interpretation of the assay results.

The first three steps may be completed in approximately two hours. The final step, to be performed on the following day, may be completed in approximately one hour.

Prior to beginning the assay, place the Hydrating Solution in a 25˚C incubator for 1 to 2 hours to ensure that it is approximately 25˚C. Remove the 24-well assay plates filled with membrane discs from the refrigerator. Allow the membrane discs to equilibrate to room temperature (approximately 30-45 minutes) before being used in Step III.D.3.

I. Instrumentation, Reagents and Materials

A. Kit Contains:
1. Dermal Reagent Powder (1 Bottle)
2. Dermal Hydrating solution (1 Bottle)
3. Dermal Blanking Buffer (1 Bottle)
4. Dermal Inhibition Check (1 Vial)
5. Dermal Activator (1 Vial)
6. Four Calibrator Solutions: Cal 0, Cal 1, Cal 2, Cal 3
7. Two Quality Control Solutions: QC 1, QC 2

8. 96-well Reading Plate (1)
9. 24-well Assay Plates Filled with Membrane Discs (26 pink discs and 22 clear discs)–**store at 2-8˚C (2)**
10. Wooden Stirring Sticks (12)
11. Whatman #1 Filter Paper, 12.5 cm diameter (1)
12. Procedural Summary
13. Range Specification Data Sheet
14. Irritection Software Instructions

B. Additional Materials Required:

1. Plate Reader (such as Modified Cambridge 7520 Microplatereader)
2. IBM compatible PC with Irritection Software
3. Incubator Maintained at 25˚C ± 1˚C
4. Balance (110 g Capacity)
5. pH Meter
6. Vortex Mixer
7. Positive Displacement Micro Pipettor (such as Labindustries Popette™ Micropipettor Model P-250, 0.5 – 250 µl)
8. Multichannel Pipettor (such as Matrix 6 Channel EXP Impact Pipettor 15-1250 µl)
9. Disposable Reservoir Trays (2) (such as 100 ml Matrix Disposable Reagent Reservoir)
10. 100 ml Graduated Cylinder
11. 100 ml Beaker
12. Funnel (such as Oxford Vented Disposable Polystyrene Funnel, 12.5 cm diameter)
13. Plastic Forceps
14. Plastic Wrap

II. Sample Preparation

A. Sample Preparation: Solids

Typically, five solid samples may be evaluated at four different concentrations. Obtain fine shavings from different locations on a solid material, e.g., a bar of soap.

1. Weigh out the required amount of sample and place it in a beaker of suitable size.
2. Add the appropriate amount of distilled water to the beaker.
3. Mix on a magnetic stir plate or use a vortex mixer to achieve dissolution of the solid.

A typical range of concentrations could be prepared according to the following scheme:

Concentration (%)	Sample Weight (g)	Volume Water (ml)
15	1.50	10.0
8	0.80	10.0
5	0.50	10.0
2	0.20	10.0
1	0.10	10.0

B. Sample Preparation: Liquids

Typically, five liquid samples may be evaluated at four different concentrations.

1. Dilute liquid samples to the desired concentrations using distilled water as the diluent.

A typical range of concentrations could be prepared according to the following scheme:

Concentration (%)	Volume Neat Sample (ml)	Volume Water (ml)
50	0.50	0.50
25	0.25	0.75
10	0.10	0.90
5	0.05	0.95
1	0.01	0.99

Note: Sample preparation should be completed prior to proceeding to Step III.

III. Procedures

A. Dermal Reagent Preparation:

1. Rehydration:
    a. Remove the Hydrating Solution from the 25˚C incubator.
    b. Using a funnel, pour the entire contents of the Hydrating Solution into the Reagent Powder bottle.
    c. Gently swirl until all of the Reagent Powder is dissolved.
    d. Let the dissolved Reagent stand at room temperature for approximately 10 minutes before filtering.

2. Filtration:
    a. Fold the filter paper and place in funnel.

b. Pour all of the dissolved Reagent into the funnel and collect the filtrate in a graduated cylinder at atmospheric pressure (do not use a vacuum pump.). This will take approximately 10 minutes.
Note: During the filtration process, label the 24-well assay plates (Step III.B).
c. Pour 40 ml of the filtered Reagent into a 100 ml beaker.
Note: The Reagent must be used within 30 minutes of rehydration.

3. Activation:
   a. Record the initial pH and temperature of the filtered Reagent, verifying that it falls within the following specified ranges: **pH: 9.79-10.49 and T: 23-25˚C.**
   b. Using the positive displacement pipettor, slowly add 920 µl of the Activator to the filtered Reagent. Gently swirl to mix.
   c. Record the final pH of the Activated Reagent solution, verifying that it falls within the following specified range: **pH: 7.48-8.36.** Note: If the pH does not fall within the specified range, recalibrate the pH meter, gently swirl the Reagent and remeasure the pH. If the pH is still not within the specified range, contact your technical representative at 1-800-2-INVITRO.
   d. Using the positive displacement pipettor, slowly add 690 µl of the Activator to the bottle of Blanking Buffer. Gently swirl to mix.
   e. It is not necessary to measure the pH or temperature of the Activated Blanking Buffer.

B. Labeling the 24-Well Assay Plates:

1. Align the assay plates with the notched corners toward the left and label their lids. A typical plate configuration that has been employed to evaluate five different samples at four different concentrations is shows in Figure 3.13 on the next page.

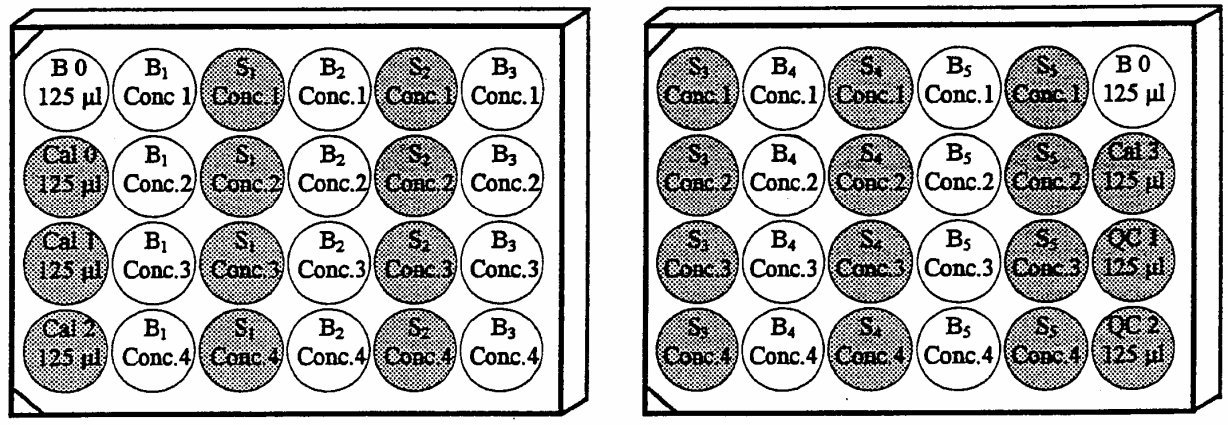


Figure 3.13. Suggested Plate Configuration: Analysis of Five Samples at Four Different Concentrations

Figure Legend:

= well contains Blanking Buffer and a clear disc

= well contains Reagent and a pink disc

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to clear membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to pink membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to pink membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to pink membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to pink membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to pink membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to pink membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 125 µl/mg of sample solution (at concentrations 1, 2, 3 and 4 respectively) applied to clear membrane discs.
S: 1.25 ml Activated Reagent in well, 125 µl/mg of sample solution (at concentrations 1, 2, 3 and 4 respectively) applied to pink membrane discs.

Note: Alternative assay schemes are shown in Figure 3.15 and 3.16 on pages 50 and 51 of this chapter.

C. Addition of Reagent and Blanking Buffer to 24-Well Assay Plates:

1. Remove the labeled lid on each assay plate and use the lid or Figure 1 as a reference when filling the wells.
2. Set the multichannel pipettor at 1250 µl.
3. Pour the Activated Reagent into a disposable reservoir tray.

4. Using the multichannel pipettor, add 1250 µl of the Activated Reagent to the following wells: Cal 0, Cal 1, Cal 2, Cal 3, QC 1, QC 2 and all wells designated for analysis of the sample (S).
5. Pour the Activated Blanking Buffer into a disposable reservoir tray.
6. Change pipette tips and add 1250 µl of the Blanking Buffer to the wells designated B 0 on both plates and all other wells designated as blanks (B).

D. Addition of Calibrators, Quality Controls and Samples:

Note: Avoid cross contamination among the separate Calibrator, Quality Control and sample wells by utilizing a clean pipette tip for each.

1. Pipette 125 µl of Cal 0 into a clear membrane disc and insert it into the B 0 well. Repeat this procedure for the B 0 well of the second assay plate,
2. Pipette 125 µl of Cal 0, Cal 1, Cal 2, Cal 3, QC 1 and QC 2 into pink membrane discs and insert them into the appropriate wells of the assay plates.
3. Pipette 125 µl of each test sample concentration into the clear and pink membrane discs and insert them into the corresponding blank and test sample wells of the assay plates.

E. Incubation:

1. To prevent evaporation of the sample from the membrane discs, tightly wrap the assay plate with plastic wrap. Place a lid on top of each wrapped plate.
2. Record the technician's name, date and time on the lids of the assay plates.
3. Place the assay plates in an incubator maintained at 25˚C ± 1˚C for 24 hours (± 30 minutes).

F. Removing Membrane Discs:

1. Remove the assay plates from the incubator.
2. Remove the lids from the assay plates and save them. They will be used as a reference when transferring the Reagent and the Blanking Buffer to the clean 96-well reading plate.
3. Remove the plastic wrap from each assay plate.
4. Remove each membrane disc individually with a plastic forceps.
5. Check the membranes for damage. Verify that all membrane discs are intact. If membrane damage has occurred, it should be recorded. Data for this sample should be carefully evaluated.
6. Using the wooden stirring sticks, scrape the Reagent wells to ensure that all of the precipitate is removed from the bottom of each well. Use one stirring stick for each sample, starting with lowest volume wells first, the progressing to wells of

higher volume. Use a separate stirring stick for each calibrator well. The wells containing Blanking Buffer do not need to be scraped.

7. Note any wells with reduced volume. Reduced cell well volumes may be indicative of hygroscopic effects or technical problems and should be recorded.

G. Transferring 250 µl of Reagent and Blanking Buffer into the 96-well Reading Plate:

1. Set the multichannel pipettor to 260 µl for filling and 250 µl for dispensing.
   Note: 260 µl is used for filling to ensure that no air bubbles are dispensed.
   The remaining 10 µl must be purged prior to refilling the multichannel pipettor.
2. Transfer 250 µl from each well of the 24-well assay plates to every other well of the 96-well reading plate by columns (see Figure 2, page 8).
3. Change pipette tips and repeat this process for each column.

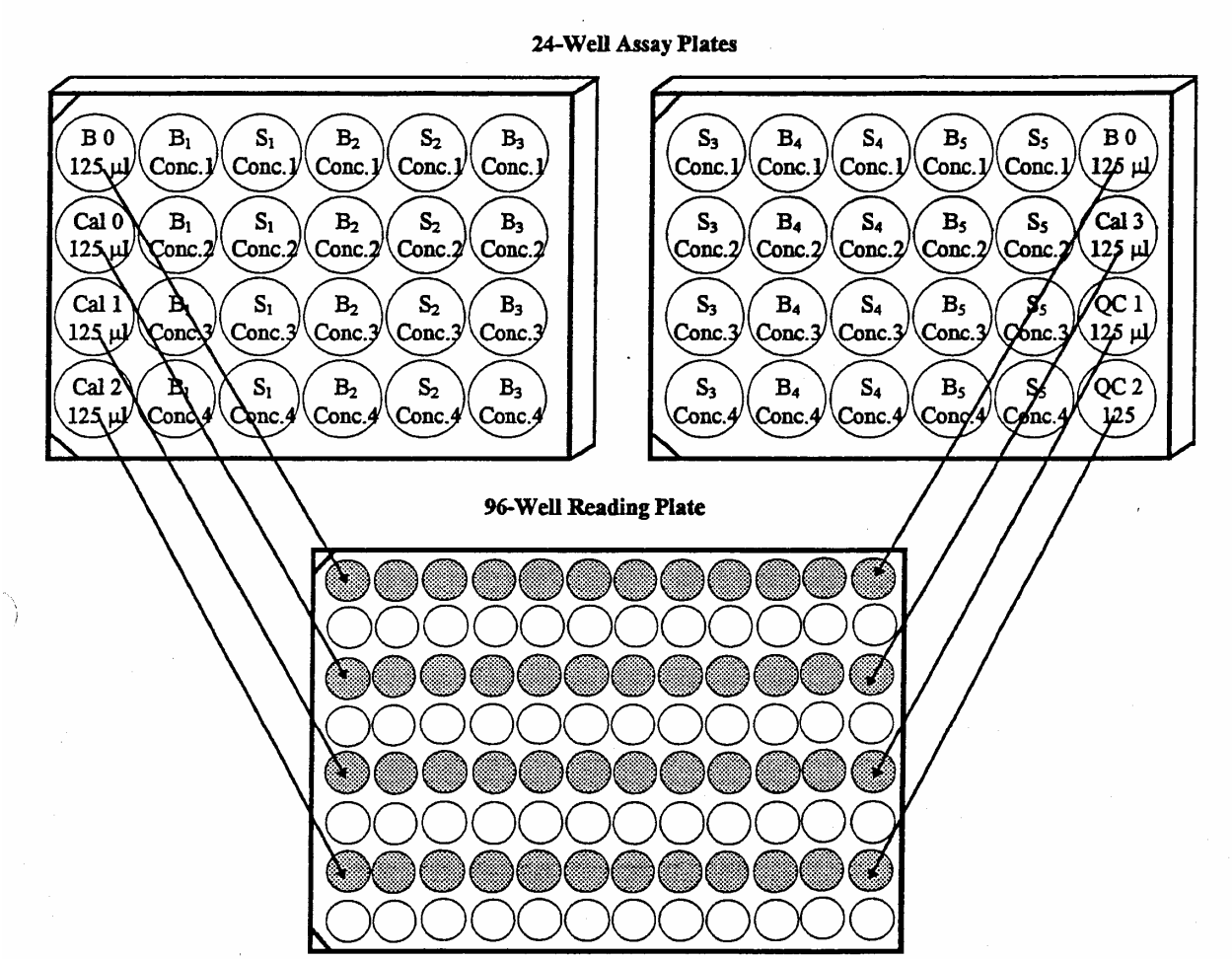


Figure 3.14. Transferring from 24-Well Assay Plates to 96-Well Reading Plate

Figure Legend:

= cell should contain 250 µl of Reagent or Blanking Buffer from the 24-well assay plates

= cell should be empty

H. Reading Assay on Plate Reader (refer to the Irritection Software Instructions found in Chapter 7 for more details:

1. To eliminate inaccurate readings produced by settling of the precipitated Reagent, the assay must be read immediately after transferring the Reagent to the reading plate.
2. If you have the Irritection Software for DOS, follow the instructions listed in Step 2a. If you have the Irritection Software for Windows™, follow the instructions listed in Step 2b.

a. Irritection Software for DOS:

(1) Ensure that the plate set up in the Irritection Software matches the configuration for the assay and that the Calibrator/Quality Control ranges in the software are updated with the current kit lot number.
(2) Select *New Assay Entry* from the *Assay* menu to initiate plate reader.
(3) Remove the reading plate lid, wipe the bottom of the plate with a Kimwipe to remove any moisture and insert the plate into the plate reader with the corner notches facing to the left.
(4) Follow screen prompts to read and analyze data **(read absorbance at 450 nm).**
(5) Upon completion of data entry, press *F10* to process data. If an Inhibition Check needs to be performed, a screen prompt will display the appropriate wells that require inhibition check.

b. Irritection Software for Windows:

(1) From the *Method* menu, choose *Select*.
(2) Select the appropriate assay method.
(3) From the *File* menu, choose *New.*
(4) Select *Assay.*
(5) Select the appropriate protocol.
(6) Select the appropriate plate layout.
(7) A series of screens will be displayed. You will need to enter the appropriate assay, and sample information.
(8) The plate reader will initialize. Remove the reading plate lid, wipe the bottom of the plate with a Kimwipe to remove any moisture and insert the plate into the plate reader with the corner notches facing to the left. Click *Continue,* or press *ENTER.*
(9) After the data collection is completed, a dialog box will be displayed indicating if the assay was qualified or unqualified. Click *OK* to continue.

(10) If an Inhibition Check needs to be performed, a screen prompt will display the appropriate wells that require Inhibition Check.

3. If an Inhibition Check is required, add 25 µl of Inhibition Check to the appropriate wells.
4. Wait five minutes, then re-read.

I. Irritection Software Qualification Checks:

The Irritection software will perform the following qualification checks to ensure assay performance.

1. Calibrator/Quality Control Check: Verifies that Calibrators/Quality Controls are within specified ranges.
2. Net Optical Density Check: Verifies that Net OD ($OD_{reagent} - OD_{blank} = OD_{Net}$) is > -15.
3. Inhibition Check: Verifies that OD of Inhibition Check is greater than OD of Cal 2.
4. Concentration Curve Check: Verifies that sample concentration curve is a parabolic curve.

J. Sample Results:

1. Upon acceptance of all qualification checks, the Irritection Software will generate a qualified assay report.
2. Human Irritancy Equivalent (HIE) scores will be calculated and a predicted *in vivo* class will be determined.
3. Customized reports with calibration and dose-response curves may be printed.

K. Clean Up:

1. The Activator, Inhibition Check and all calibrators should be disposed of after the assay has been completed. See MSD Sheets for proper disposal procedures.

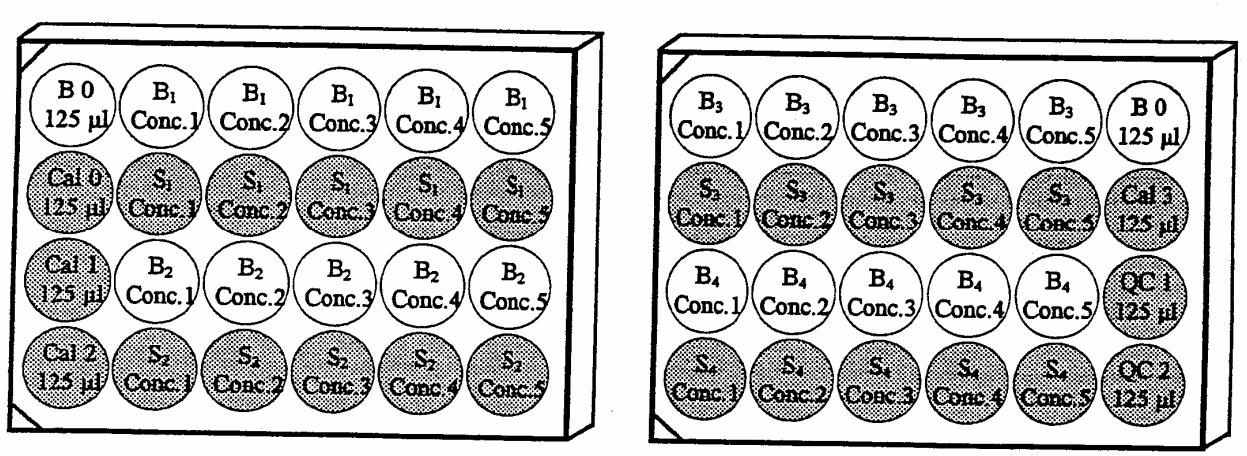


Figure 3.15. Plate Configuration – Alternative I
Suitable for Four Samples at Five Concentrations

Figure Legend:

= well contains Blanking Buffer and a clear disc

= well contains Reagent and a pink disc

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to clear membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to pink membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to pink membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to pink membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to pink membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to pink membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to pink membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 125 µl of sample (at concentration 1, 2, 3, 4 and 5, respectively) applied to clear membrane disc.
S: 1.25 ml Activated Reagent in well, 125 µl of sample (at concentration 1, 2, 3, 4 and 5, respectively)applied to pink membrane disc.

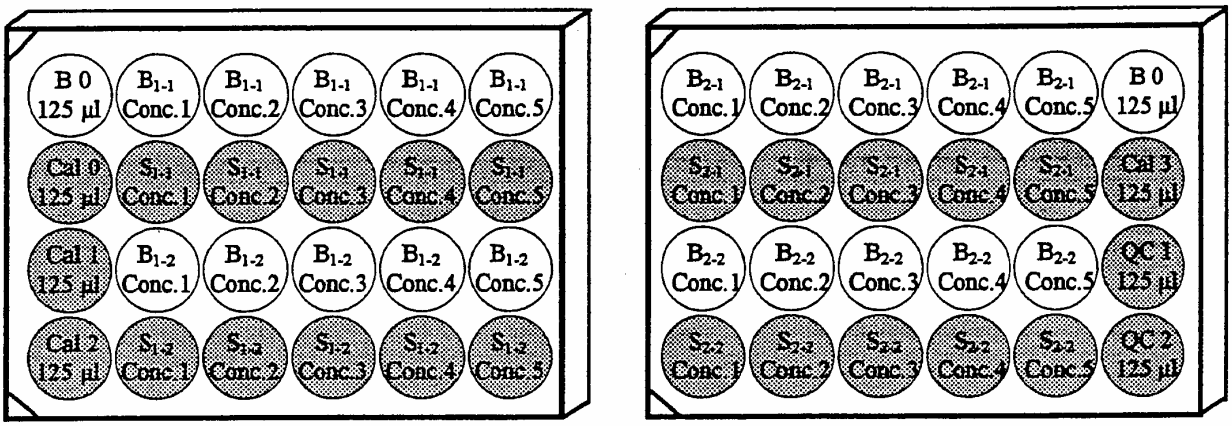


Figure 3.16. Plate Configuration – Alternative II
Suitable for Two Samples at Five Concentrations in Duplicates

Figure Legend:

= well contains Blanking Buffer and a clear disc

= well contains Reagent and a pink disc

B 0: 1.25 ml Activated Blanking Buffer in well, 125 µl of Cal 0 applied to clear membrane disc.
Cal 0: 1.25 ml Activated Reagent in well, 125 µl of Cal 0 applied to pink membrane disc.
Cal 1: 1.25 ml Activated Reagent in well, 125 µl of Cal 1 applied to pink membrane disc.
Cal 2: 1.25 ml Activated Reagent in well, 125 µl of Cal 2 applied to pink membrane disc.
Cal 3: 1.25 ml Activated Reagent in well, 125 µl of Cal 3 applied to pink membrane disc.
QC 1: 1.25 ml Activated Reagent in well, 125 µl of QC 1 applied to pink membrane disc.
QC 2: 1.25 ml Activated Reagent in well, 125 µl of QC 2 applied to pink membrane disc.
B: 1.25 ml Activated Blanking Buffer in well, 25 µl of sample (at concentrations 1, 2, 3, 4 and 5, respectively) applied to membrane discs.
S: 1.25 ml Activated Reagent in well, 125 µl of sample (at concentrations 1, 2, 3, 4 and 5, respectively) applied to pink membrane discs.

Irritection® Assay System

Instruction Manual

Chapter 4
Results of Irritection Studies

InVitro International
17751 Sky Park East, Suite G
Irvine, California, 92614

The initial evaluation of the performance of the Irritection Assay system was conducted by performing a series of studies with defined irritants. Both Ocular and Dermal assays were assessed in this fashion. In each case, as noted in the legends, volume-dependent dose-response studies were performed. The results of these studies are summarized in Table 4.1 and figure 4.1 – 4.3 found on the following pages.

These investigations revealed at least tow important phenomena. First, it was noted that, as a general rule, the maximal IDE or HIE score was observed when the maximal volume (125 µl) of irritant was applied to the sample delivery membrane disc. This IDE/HIE value, termed the Maximum Qualified Score (MQS), serves as an important means of characterizing the irritancy potential of the test material. Methods of interpreting these types of dose-response curves, and defining the resultant MQS are covered in greater detail in Chapter 5 of this manual. Second, the data clearly demonstrated that both the Ocular and Dermal assays were highly reproducible. For example, when assessed at the maximum volume applied, mild, moderate, and severe irritants typically displayed a 5 to 15% variation from their mean value.

Taken together, these findings demonstrated that both the Ocular and Dermal Irritection assays could be employed to reliably define the irritancy potential of a wide variety of irritant materials.

Ocular Cosmetic Assay Results

Volume Applied (µl)	Minimal Irritant IDE Scores (N=13)			Mild Irritant IDE Scores (N=13)			Moderate Irritant IDE Scores (N=13)			Severe Irritant IDE Scores (N=13)		
	Mean	±SD	±%SD	Mean	±SD	±%SD	Mean	±SD	±%SD	Mean	±SD	±%SD
25	7.88	3.41	43.3	9.40	3.95	42.0	18.75	5.43	29.0	38.74	5.43	14.0
50	5.94	1.18	19.9	11.68	4.02	34.4	24.82	3.87	15.6	50.29	1.28	2.5
75	5.48	1.30	23.7	15.34	1.71	11.1	29.03	4.29	14.8	50.50	1.13	2.2
100	5.54	0.63	11.4	17.04	0.95	5.6	32.40	3.58	11.0	50.92	0.23	0.5
125	5.50	1.00	18.2	19.35	0.96	5.0	32.47	3.96	12.2	50.70	0.92	1.8

Ocular Surfactant (5 Hour) Assay Results

Volume Applied (µl)	Minimal Irritant IDE Scores (N=13)			Mild Irritant IDE Scores (N=13)			Moderate Irritant IDE Scores (N=13)			Severe Irritant IDE Scores (N=13)		
	Mean	±SD	±%SD	Mean	±SD	±%SD	Mean	±SD	±%SD	Mean	±SD	±%SD
25	8.34	2.54	30.5	12.76	2.41	18.9	16.89	4.42	26.2	35.79	3.07	8.6
50	5.36	1.84	34.3	13.71	1.58	11.5	19.79	4.55	23.0	47.88	3.24	6.8
75	4.17	1.67	40.0	15.69	1.84	11.7	23.26	5.48	23.6	49.91	1.63	3.3
100	4.08	1.90	46.6	17.69	2.07	11.7	25.45	4.71	18.5	50.11	1.42	2.8
125	4.26	1.52	35.7	20.26	1.93	9.5	27.20	4.02	14.8	29.94	1.87	3.7

Dermal Cosmetic Assay Results

Volume Applied (µl)	Minimal Irritant IDE Scores (N=13)			Mild Irritant IDE Scores (N=13)			Moderate Irritant IDE Scores (N=13)			Severe Irritant IDE Scores (N=13)		
	Mean	±SD	±%SD	Mean	±SD	±%SD	Mean	±SD	±%SD	Mean	±SD	±%SD
25	0.35	0.20	57.1	0.25	0.11	44.0	0.43	0.21	48.8	1.23	0.60	48.8
50	0.21	0.15	71.4	0.30	0.10	33.3	0.63	0.32	50.8	3.02	0.80	26.5
75	0.22	0.19	86.4	0.38	0.09	23.7	1.64	0.48	29.3	3.29	0.45	13.7
100	0.18	0.12	66.7	0.44	0.09	20.5	2.17	0.44	20.3	3.52	0.60	17.0
125	0.20	0.17	85.0	0.59	0.10	16.9	2.37	0.30	12.7	3.42	0.50	14.6

Table 4.1. Variability of Ocular and Dermal Irritection Assay Results with Defined Irritants

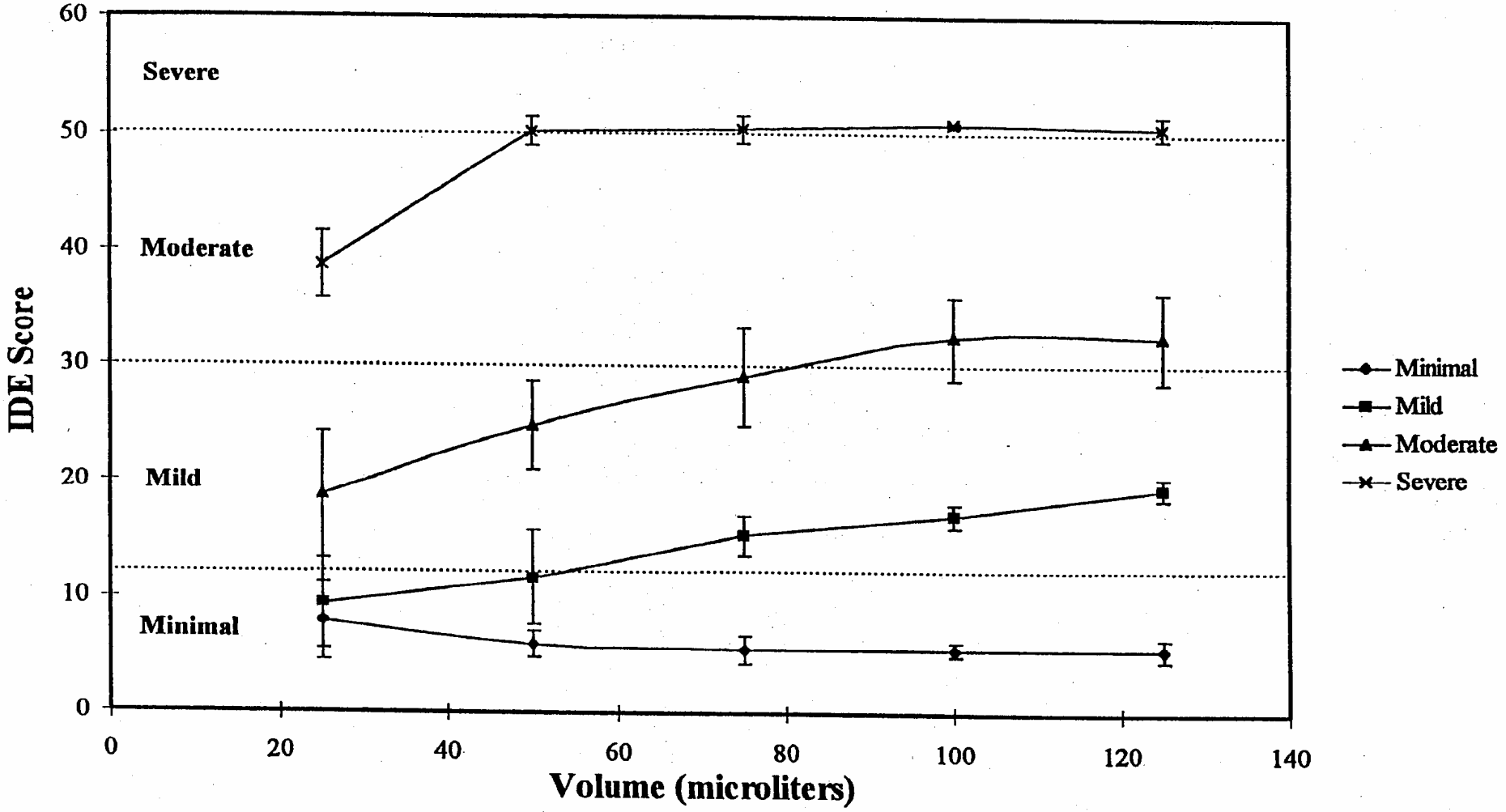


Figure 4.1. Variability of Ocular Irritection Assay with Defined Irritants

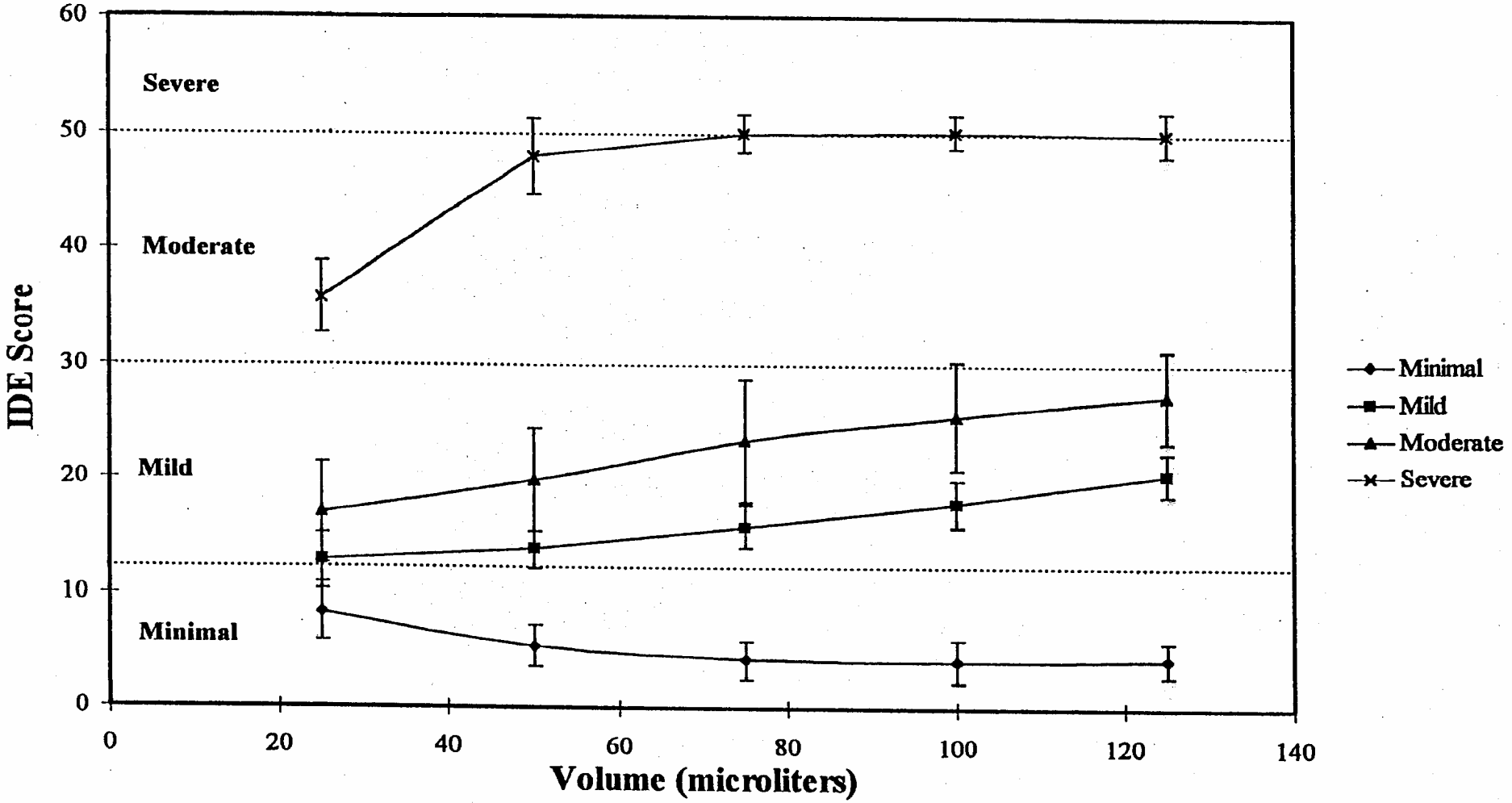


Figure 4.2. Variability of Ocular Irritection Assay (5 Hour Surfactant Protocol) with Defined Irritants

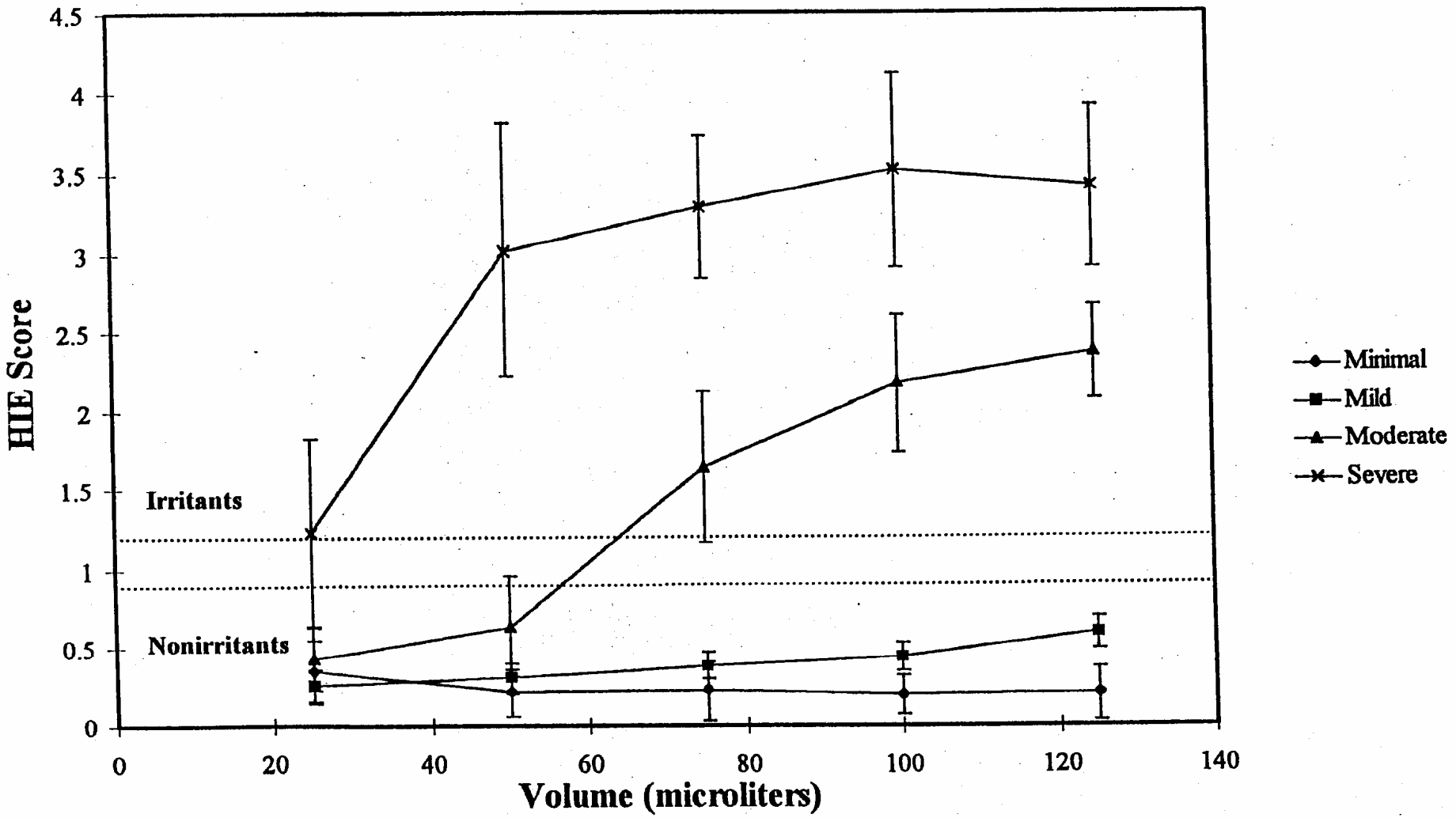


Figure 4.3. Variability of Dermal Irritection Assay with Defined Irritants

Following completion of the internal evaluation of the Irritection Assay System, additional studies were performed by conducting a series of inter-laboratory comparative studies utilizing a set of reference chemicals. The primary objectives of these investigations were three-fold. First, to demonstrate that the Ocular Irritection assays were applicable over a broad range. Second, to confirm that the results obtained with the Irritection assays were similar to those obtained with the Eytex test (Chapter 2). Third, to define the inter-laboratory variability of the *in vitro* tests.

The results of these investigations are summarized in Table 4.2 on the next page. These findings may be briefly summarized as follows:

- The Ocular Irritection assays detect and distinguish the irritancy of chemicals whose *in vivo* classification ranged from minimal to severe.

- The Irritection and Eytex assays provided comparable results as judged by both their equivalence score (95%) and the correlation coefficient calculated by linear regression analysis (mean r = 0.98).

- The Irritection test was found to display a somewhat better correlation with the results of the Draize test (equivalence = 87.5%) than the Eytex test did (equivalence = 81.3%).

- The inter-laboratory variance for the Irritection test was quite acceptable, being calculated to be 13.9% of the mean for this group of reference chemicals.

Taken together, these observations confirm that the Ocular Irritection Assays provide results that correlate closely with those obtained *in vivo* and are somewhat more reliable than those provided by the earlier Eytex test.

These initial findings were subsequently confirmed and extended in a second study that was performed with a much larger group of chemicals. The results of this investigation are summarized in Table 4.3. The 2X2 contingency table presented here summarizes the frequency of observations of agreement and disagreement between the Ocular Irritection test method and the Draize test method and permits calculation of the following comparative parameters:

- Equivalence of Irritection and Draize Assays = 83.0%
- Sensitivity (Irritection Method Correctly Identifies Irritants) = 86.4%
- Specificity (Irritection Method Correctly Identifies Non-Irritants) = 93.5%
- Predictive Value (Irritection Method Correctly Predicts Irritancy) = 90.5%

		Draize Method	
		Non-Irritants	**Irritants**
Irritection Method	**Non-Irritants**	29	3
	Irritants	2	19

Sample	Irritection® Data						Eytex® Data						*In Vivo* Classification
	A	B	C	D	Mean	Class	A	B	C	D	Mean	Class	
0.1% SDC	23.1	24.1	23.7	17.6	22.1	Mild/Mod	19.4	16.6	13.7	16.4	16.5	Min/Mild	
1% BAS	38.2	36.6	42.9	36.3	38.5	Severe	42.6	>51	39.2	38.8	40.2	Sever	Severe
1% Acetic Acid	29.7	27.3	24.7	26.1	27.0	Mod	22.9	22.8	25.3	23.9	23.7	Mild/Mod	
10% Acetic Acid	46.9	47.5	48.6	49.3	48.1	Sev/Extr	47.1	49.0		46.1	47.4	Sev/Extr	Severe
Ethylene Glycol	4.4	NQ	1.9	3.9	3.4	Min	5.1	NQ	4.2	NQ	4.7	Min	Min
Propylene Glycol	7.4	7.7	4.3	7.0	6.6	Min	8.8	8.3	7.1	5.7	7.5	Min	Min
NaC1	14.4	22.5	14.2	18.5	17.4	Min/Mild	14.4	14.7		15.6	14.9	Min	Mild
Methanol	22.8	26.4	21.4	20.5	22.8	Mild/Mod	25.0	18.4	25.8	26.6	24.0	Mild/Mod	Mild/Mod
Triacetin	30.4	31.8	28.1	28.4	29.7	Mod	30.3	25.3	27.9	25.0	27.1	Mod	Min
n-Propanol	35.0	32.8	33.8	31.3	33.2	Mod/Sev	37.0	32.2		29.7	33.0	Mod/Sev	Mod

Inter-Laboratory Correlations

Laboratory	Irritection® : Eytex® (r)	Irritection® : Irritection® (r)	Eytex® : Eytex® (r)
A	0.97		
B	0.95		
C	0.94		
D	0.97		
Mean Four Laboratories	0.98		
A:B		0.96	0.97
A:C		0.99	0.98
A:D		0.98	0.97

Equivalence Scores

Irritection®: *In Vivo* = 87.5%

Irritection® : Eytex® = 95%

Eytex®: *In Vivo* = 81.3%

Table 4.2 Comparison of Ocular Irritection®, Eytex® and *In Vivo* Measures of Irritancy

Chemical	Irritection Classification	*In vivo* Classification	Equivalence	Irritection Score > 25	*In Vivo* >Mild/Mod	Equivalence
1-Butanol	Mod/Sev	Sev/Ext	1	+	+	+
2,4-Dichlorophenoxyacetic Acid	Mod/Sev	Sev/Ext	1	+	+	+
2,5-Hexanediol	Mild	Min	0.5	-	-	-
2-Butoxyethyl Acetate	Min	Min/Mild	1	-	-	-
2-Methoxyethanol	Mild	Min	0.5	-	-	-
Acetic Acid, 10%	Mod/Sev	Sev/Ext	1	+	+	+
Acetonitrile	Mild	Mod	0.5	+	+	+
Acrolein	Mild	Sev/Ext	0	-	+	0
Acrylamide	Mild/Mod	Mod	1	+	+	+
Acrylonitrile	Mild	Mild	1	-	-	-
Aniline	Mod/Sev	Mod	1	+	+	+
Benzalkonium Chloride, 10%	Mod/Sev	Sev/Ext	1	+	+	+
Benzalkonium Chloride, 10%	Mild/Mod	Sev/Ext	0.5	+	+	+
Betaine	Min/Mild	Mild	1	-	-	-
Boric Acid, 1%	Min/Mild	Mild/Mod	1	-	-	-
Brij 35	Min	Min	1	-	-	-
CHAPSO, 1%	Mild	Min	0.5	-	-	-
Chloramphenicol, 1%	Min	Min	1	-	-	-
Chloroform	Mild	Mod	0.5	-	+	0
Chlorpromazine HCI, 1%	Mild/Mod	Mod	1	+	+	+
Citric Acid	Mod/Sev	Sev/Ext	1	+	+	+
Crystal Violet, 10%	Mild/Mod	Mod/Sev	0.5	+	+	+
Cycloheximide	Mild	MinMild	1	-	-	-
Dibutyltin Dichloride	Mod/Sev	Mod/Sev	1	+	+	+
DMSO	Mild	Min	0.5	-	-	-

Table 4.3. Summary of Ocular Irritection® Assay System Results

Chemical	Irritection Classification	*In vivo* Classification	Equivalence	Irritection Score > 25	*In Vivo* >Mild/Mod	Equivalence
EDTA	Mod/Sev	Sev/Ext	1	+	+	+
Ethanol	Mild	Mild/Mod	1	-	-	-
Ethylene Glycol	Min	Min	1	-	-	-
Fluorescein, 10%	Min/Mild	Mild	1	-	-	-
Glycerol	Min	Min	1	-	-	-
Hexane	Mild	Min	0.5	-	-	-
Menthol	Min/Mild	Sev/Ext	0	-	+	0
Propylene Glycol	Min	Min	1	-	-	-
Silver Nitate, 5%	Min/Mild	Min	1	-	-	-
Sodium Chloride, 0.9%	Mild	Mild	1	-	-	-
Sodium Dodecyl Sulfate, 30%	Mild/Mod	Mod	1	+	+	+
Sodium Hydroxide, 10%	Mild/Mod	Mod/Sev	0	+	+	+
Sodium Stearate	Min	Min	1	-	-	-
Squalene	Min	Min	1	-	-	-
Strearic Acid	Min	Min	1	-	-	-
Talc	Min/Mild	Min	1	-	-	-
Toluene	Mild	Mild	1	-	-	-
Triacetin	Mild	Min	0.5	+	-	0
Tributyltin Chloride	Mod	Sev/Ext	0.5	+	+	+
Triethanol Amine	Mod	Mild/Mod	1	+	-	0
Triton X-100	Min/Mild	Min	1	-	-	-
Tween 80, 10%	Min/Mild	Min	1	-	-	-

Table 4.3 (Continued)

As noted previously, surfactants and surfactant-containing compounds exhibit a unique behavior when analyzed in the Irritection Assay System. Consequently, they are most accurately characterized by utilizing them to perform concentration-dependent studies. This observation has been confirmed by conducting these types of investigations with a representative sample of commonly used surfactants. The results of these studies are summarized in Table 4.4 and Figure 4.4 shown on the next page.

Surfactant Type	Sample Number	Draize Score	Irritection Assay Score[a,b]
Amphoteric	29	20.7	13.1
Amphoteric	35	5.3	18.6
Amphoteric	79	4.0	16.2
Anionic	27	34.3	36.9
Anionic	36	33.0	38.1
Cationic	25	0.67	10.5
Cationic	30	14.0	11.1
Cationic	31	3.3	11.8
Blends	44	12.0	17.9
Blends	46	18.0	17.3
Blends	69	18.0	17.3
Correlation:		**Vs Draize**	**R = 0.82** **y = 0.68x + 8.8**

Table 4.4. Summary of Irritection Results Obtained with Surfactants

[a] Score = Maximal Score Observed with Qualified Dose-Response Curve

[b] Concentration-Dependent Assays Performed by Applying 125 µl of Surfactant; Concentrations Tested: 0.1, 0.3, 1.0, 3.0, and 10% (v/v).

These studies demonstrate two important findings. First, as indicated by the calculated correlation coefficient of 0.80, the irritancy scores determined with the Irritection method are highly correlated with those determined *in vivo*. Second, this correlation is true for a wide variety of surfactants, with the exception that the Irritection method appears to over estimate the irritancy of nonionic surfactants.

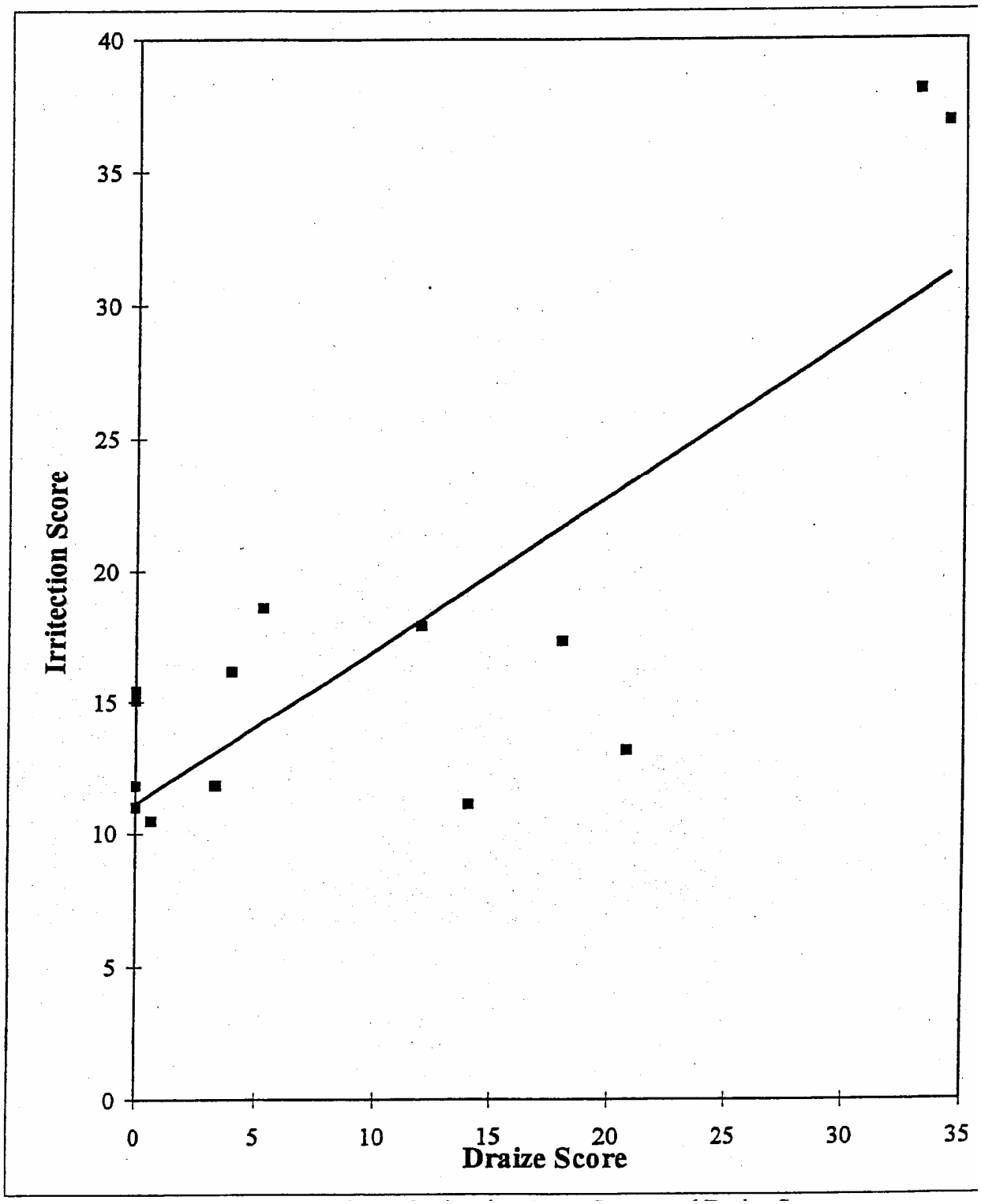


Figure 4.4. Comparison of Irritection Assay Scores and Draize Scores

The accuracy and reproducibility of the Dermal version of the Irritection Assay System has been evaluated in a blinded study of eight liquid dishwashing detergents whose dermal irritancy properties had previously been defined by accepted human dermal patch testing methods. These investigations also compared results obtained with the Irritection test method to those obtained with the earlier Skintex test.

The Dermal Irritection assay was performed on four separate occasions by three different laboratory scientists in order to both characterize the samples and provide a mean of accessing the reproducibility of this particular assay method. The results of these studies are summarized in Figure 4.5 below.

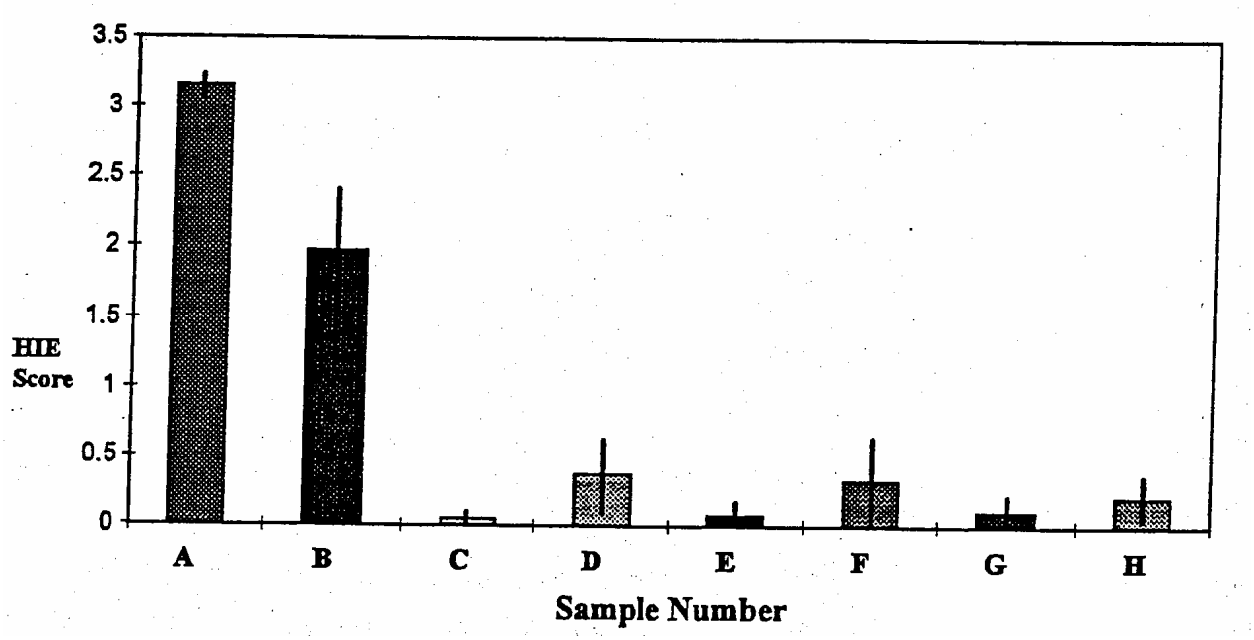


Figure 4.5. Dermal Irritection Assay Results Obtained for 10% Solutions of Liquid Dishwashing Detergents. Results are Expressed as Mean Value ± Standard Deviation.

These findings demonstrate the two of the formulations would be predicted to be irritant and the remaining six would be considered to be non-irritating. Further, these investigations demonstrated that the Dermal Irritection test was highly reproducible, with irritant detergents displaying irritancy scores whose standard deviation was only 3% of the mean score value. Of equal importance, the Dermal Irritection test method accurately predicted the dermal irritancy of all eight detergents when the *in vitro* test scores were compared to the *in vivo* findings. The Skintex test method was also found to be capable of discriminating irritant from non-irritant detergents. However, the results of the Skintex tests and *in vivo* tests were concordant for only seven of the eight detergents evaluated. These findings indicate that the Dermal Irritection test is an accurate and reproducible *in vitro* method of predicting the dermal irritancy potential of liquid detergents. As such, it may be successfully employed as a rapid screening tool to facilitate new product development and formulation activities.

Irritection® Assay System

Instruction Manual

Chapter 5
Interpretation of Data

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The windows™ software provided with the Irritection Assay System performs four important functions. They are as follows:

- Compilation of the spectrophotometric reading obtained at the completion of the assay procedure,
- Comparison of the results obtained with the test samples to those obtained with a series of calibration (Cal) and quality control (QC) standards,
- Calculation of the irritancy scores for each test material, and
- Presentation of the assay information in tabular and graphic form in a four-page print out.

In general, the computer program print out provides a clear interpretation of the experimental results. The **Sample Results** section of the report lists the optical density data, calculated irritancy scores, and irritancy classifications based on the correlative information summarized in Tables 5.1 and 5.2 below.

Irritection Draize Equivalent (IDE)	Predicted Ocular Irritancy Classification
0.0 – 12.5	Minimal Irritant
12.5 – 30.0	Mild Irritant
30.0 – 51.0	Moderate Irritant
51.0 – 80.0	Severe Irritant

Table 5.1 Relationship of Irritection Draize Equivalent (IDE) Score to Irritancy Classification for the Ocular Irritection Test Method

Human Irritancy Equivalent (HIE)	Predicted Dermal Irritancy Classification
0.00 – 0.90	Non-Irritant
0.90 – 1.20	Non-Irritant/Irritant
1.20 – 5.00	Irritant

Table 5.2 Relationship of Human Irritancy Equivalent (HIE) Score to Irritancy Classification for the Dermal Irritection Test Method

Prior investigations have demonstrated that the irritancy score (Ocular Irritection Draize Equivalent [IDE] or Dermal Human Irritancy Equivalent [HIE]) that correlates most closely with the *in vivo* irritancy properties of a test material is the highest qualified score calculated by the Irritection software. In the discussion that follows, this value has been defined as the Maximum Qualified Score (MQS). Examples of test results with the MQS denoted in each case are included in Figure 5.1 to 5.4.

The **Sample Results** section of the assay report also contains a statement about the "Qualification" of the test material at each tested volume or concentration. In most cases, this column of the report will contain a series of statements indicating that each sample tested was "Qualified". This statement simply means that the computer software has utilized an internal algorithm to analyze the data for each dose tested and found that the results obey the expected behavior of typical dose-repose curve.

Occasionally, the Qualification section of the report will contain a statement directing the user to "Examine Dose Curve" or "Examine Concentration Curve." These messages are printed when the computer algorithm detects an unexpected assay result. The primary purpose of these messages is to cause the user to examine the printed graph of the test results to determine the source of the unexpected behavior.

In general, two phenomena have been identified as producing atypical dose-response profiles and serving as potential sources of these messages. Most commonly, the "Examine Dose Curve" message is printed when the volume-dependent dose-response protocol is employed and the tested material contains a surfactant which produces a declining, rather than increasing, dose-response profile. The other common source of the "Examine Dose or Concentration Curve" message is an artifact that results in an unusually elevated value being detected. This type of artifact most commonly results from a pin-hole leak in the membrane delivery device or because the tested material was very hygroscopic.

Additionally it should be noted that the "Examine Concentration Curve" message will not be printed if the concentration-dependent dose-response (surfactant) protocol is employed and the software's curve fitting program is enabled. This results from the fact that the Irritection algorithm accommodates a declining dose-response profile when analyzing known surfactant.

The following figures are provided as examples of typical volume-dependent and concentration-dependent assay profiles. They should be employed to assist in the interpretation of common assay findings.

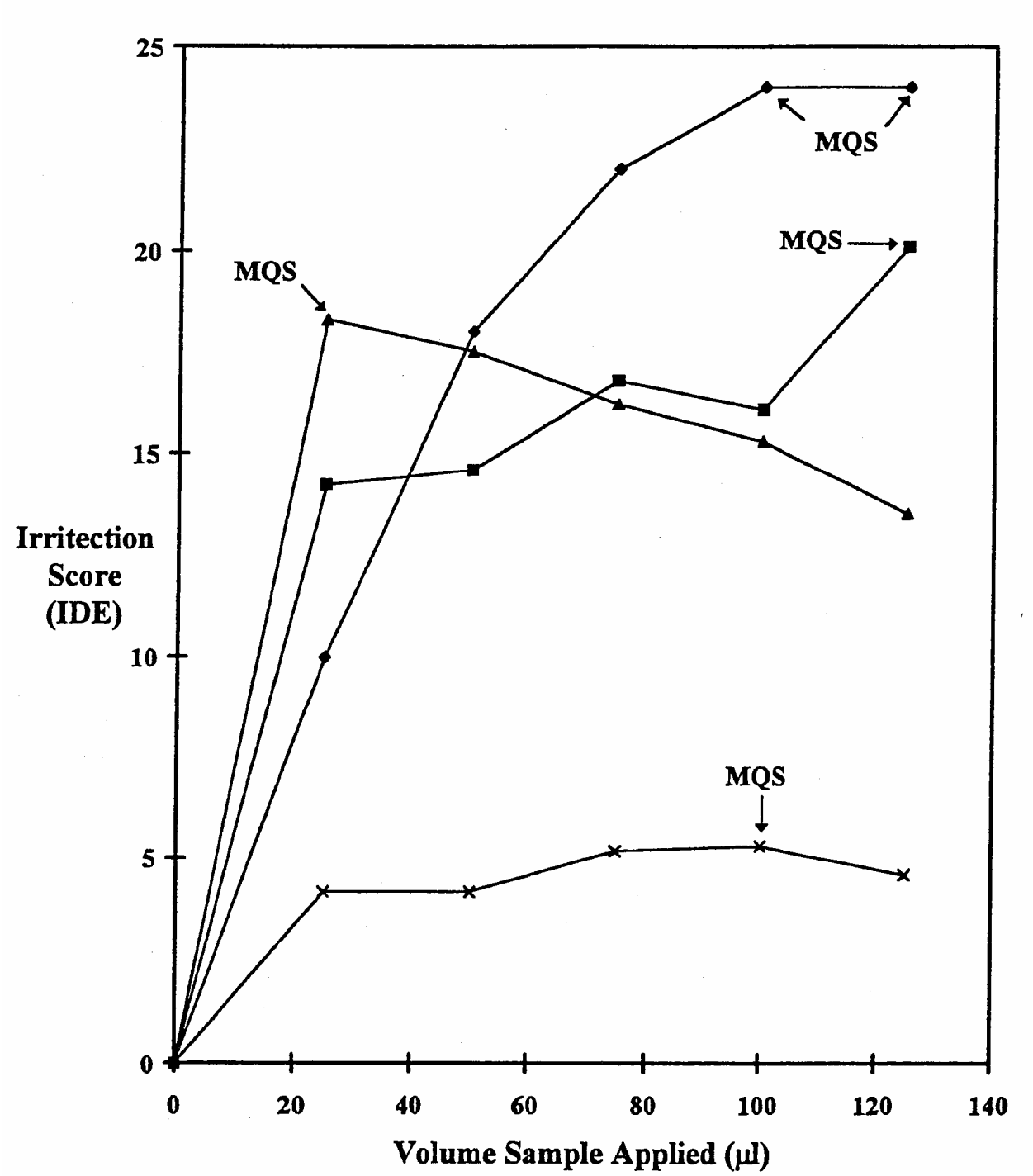

Figure 5.1 Examples of Typical Ocular Volume-Dependent Dose-Response Profiles

The Irritection software print out would report the test materials denoted by the symbols (■), (◆) and (⧖) to be “Qualified." By contrast, the material designated by the symbol (◆) displays a decreasing dose-response profile and the Qualification message would read, “Examine Dose Curve.”

Maximum Qualified Scores (MQS) are denoted in each case.

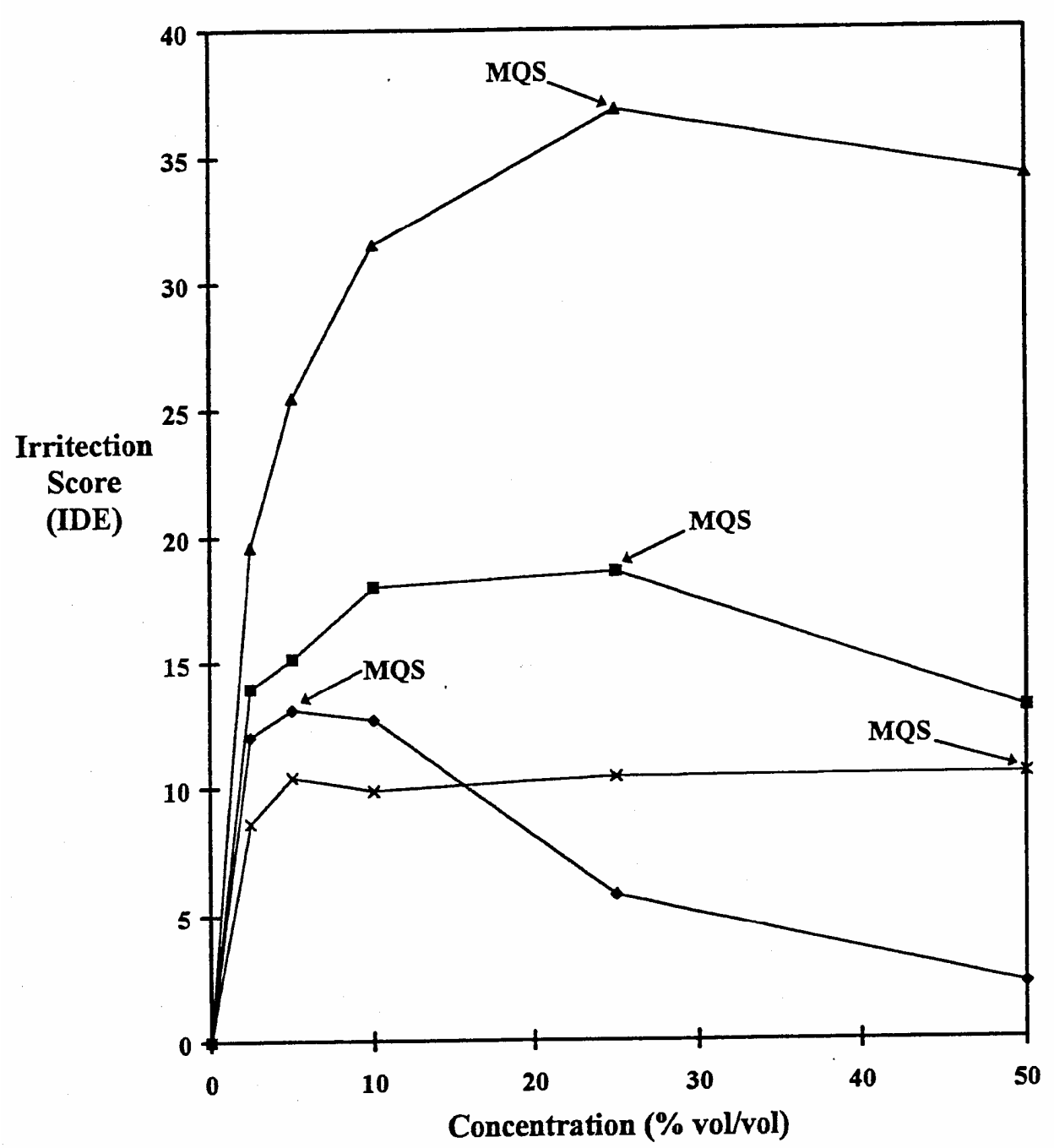

Figure 5.2 Examples of Typical Ocular Concentration-Dependent Dose-Response Profiles

The Irritection software print out would report the test materials denoted by the symbols (■), (◆) and (⧖) to be "Qualified." Additionally, the surfactant designated by the symbol (◆), which displays a decreasing dose-response profile, would be reported as qualified, **if the curve fitting**

program had been enabled. If this software feature had not been enabled, the Qualification message would have read, "Examine Concentration Curve."

Maximum Qualified Scores (MQS) are denoted in each case.

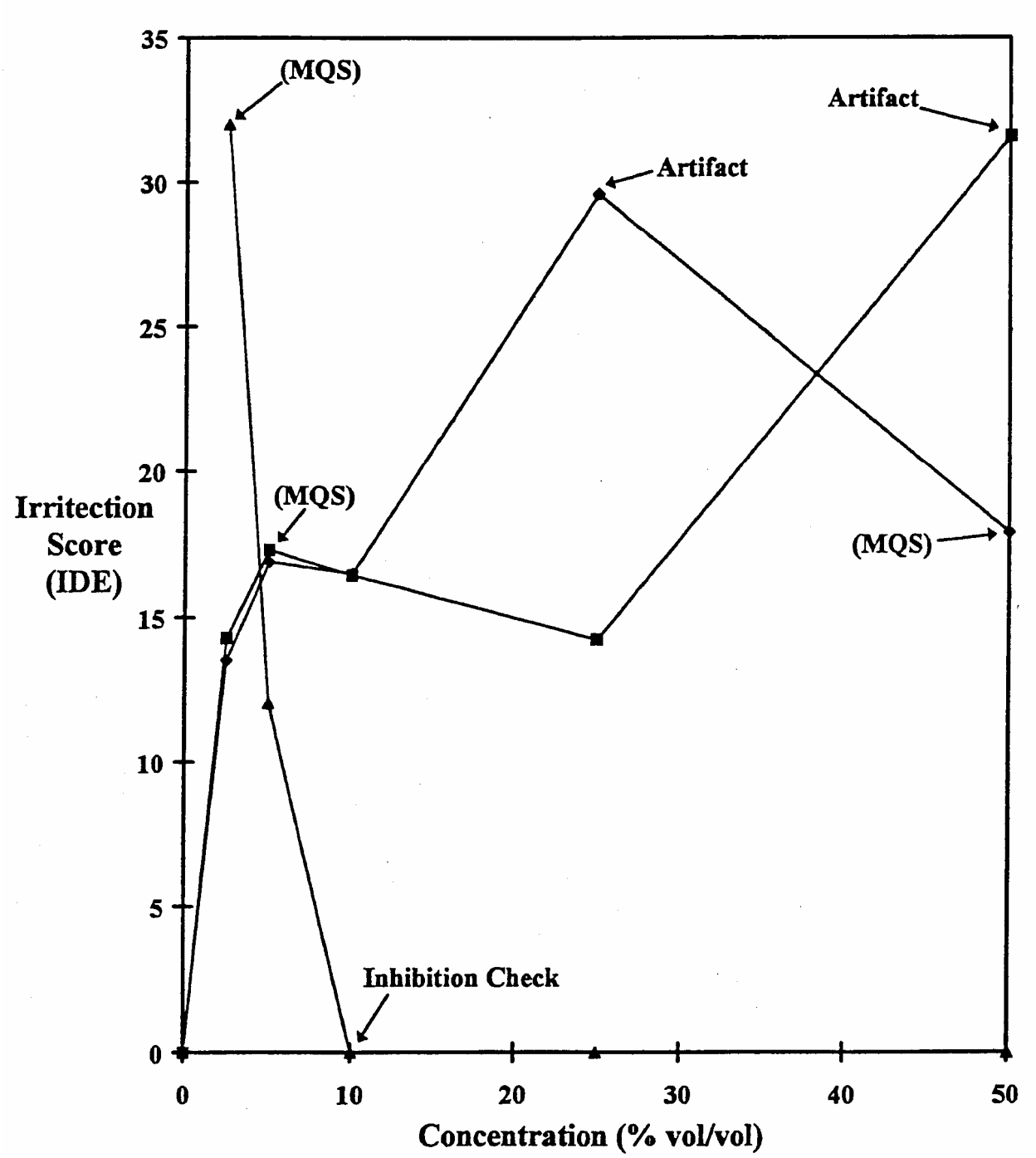

Figure 5.3 Examples of Typical Ocular Concentration-Dependent Dose-Response Profiles

The Irritection software print out would report a Qualification message reading, "Examine Concentration Curve" for all of the test materials. The material denoted by the symbol (◆) is a

surfactant that displays a dramatically decreasing dose-response profile. Additionally, this material would interfere with the inhibition check portion of the assay. Artifacts denote anomalously elevated IDE scores.

Maximum Qualified Scores (MQS) are denoted in each case.

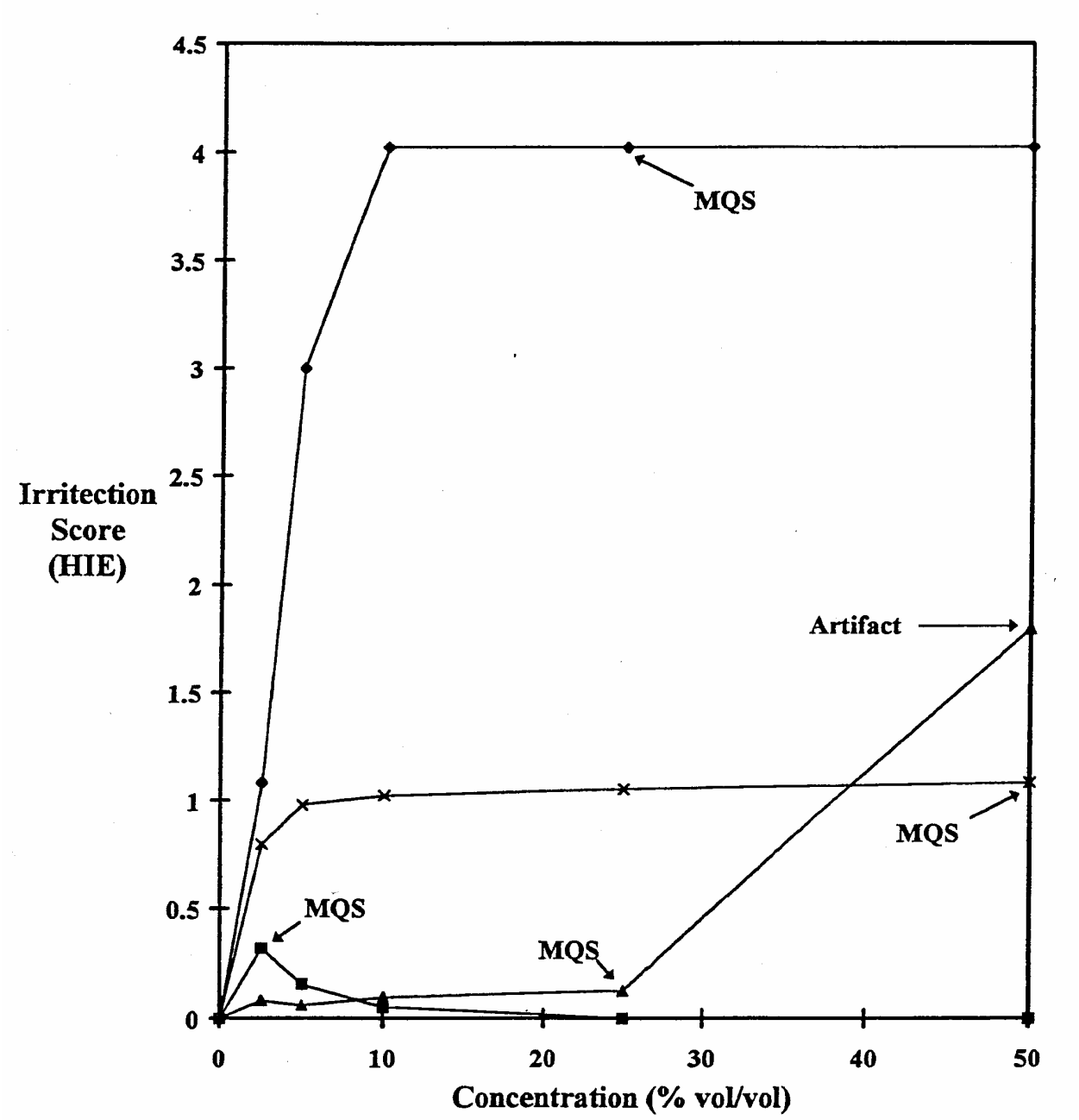

Figure 5.4 Examples of Typical Dermal Concentration-Dependent Dose-Response Profiles

The Irritection software print out would report the test materials denoted by the symbols (■), (◆) and (⧖) to be “Qualified," if the curve fitting program is enabled. By contrast, the curve containing the artifact in the profile denoted by the symbol (◆) would produce a Qualification message reading, “Examine Concentration Curve.”

Maximum Qualified Scores (MQS) are denoted in each case.

Irritection® Assay System

Instruction Manual

Chapter 6
Incompatible Compounds

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Certain types of chemicals and chemical formulations are known to be incompatible with the protein reagents that are utilized in the Ocular and Dermal Irritection assays. These materials are described below in Table 6.1.

Table 6.1 Materials that are Incompatible with The Irritection Assay System

Chemical or Chemical Formulation	Nature of Incompatibility	Solution for Problem
Acidic formulations, pH<2.0	False negative or precipitation	Dilute or neutralize sample
Alkaline formulations, pH>9.0	False negative	Dilute or neutralize sample
Intensely colored materials	High OD readings for blanks and samples	Dilute sample or read OD at 700 nm
Oils and water-insoluble organic chemicals	Oil droplets may produce erratic dose-response profiles	
Volatile ketones	Evaporation results in an under-estimation of irritancy	
Nonionic Surfactants	False positive over-estimates of irritancy	
Sorbitol at concentrations>5%	False positive	Dilute Sample
Urea at concentrations>5%	False negative	Dilute Sample
Manganese violet	False positive	Dilute Sample
Aluminum chlorohydrate	False positive	Dilute Sample with propylene glycol
Aluminum zirconium chlorohydrate	False positive	Dilute Sample with propylene glycol
Aluminum chloride	False positive	Dilute Sample with propylene glycol
Titanium oxide	False positive	Dilute Sample
Zinc oxide	False positive	Dilute Sample
Silver salts	False positive	Dilute Sample
Ferrous sulfate	False positive	Dilute Sample
Zink sulfate	False positive	Dilute Sample

Irritection® Assay System

Instruction Manual

Chapter 7
Irritection Computer Software Instructions

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IRRITERCTION® SOFTWARE INSTRUCTIONS FOR WINDOWS

A. Installing the Irritection Software

1. Insert the Irritection Software installation disk into the 3.5" disk drive.
2. From the Program Manager, go to the *File* menu, and select *Run.*
3. A Run dialog box will be displayed. Click on the *Browse* button.
4. From the Driver box, click the 3.5" disk drive (*a* or *b*). From the File Name Box, click the *setup.exe* fine, then click the *OK* button.
5. A Run dialog box will be displayed. Click the *OK* button.
6. The Irritection Software Setup will begin initialization. Click on the *Continue* button.
7. A Destination Path dialog box will be displayed. Click on the *Continue* button.
8. After the software has been installed, a message will appear stating, "Setup Succeeded." Click the *OK* button.
   Note: If the installation was not successful, please call your Technical Representative for assistance.

B. Setting Up the Instrument

1. Double-click on the Irritection Software icon to launch the program.
2. From the *Instrument* menu, choose *Setup.*
   You will need to enter the following system parameters (this needs to only be done once):

   Comm Port:
   Select the appropriate Comm Port.

   Filters (nm):
   The wavelength of each filter installed on the filter wheel of the plate reader will need to be entered in order for the program to choose the correct filter when reading. Enter zero to indicate an empty position.

   Serial Number:
   The serial number of the plate reader will need to be entered.
   Note: For the Cambridge plate reader, the serial number can be found inside the plate reader on the left corner panel. For the Molecular Devices or Dynatech plate reader, the serial number can be found on the rear panel.

   Instrument Type:
   Click the arrow in the Instrument Type box, and select the appropriate instrument type.
   Note: The *Baud Rate, Data Bits, Parity, and Stop Bits* will correctly default for each instrument type. The *Plate Type* will default on Generic 96-Well.

3. Click the *OK* button, or press *ENTER.*

C. Selecting the Assay Method

1. From the *Method* menu, choose *Select.*
2. Select the desired assay method (Ocular, High Sensitivity Ocular, Dermal, or High Sensitivity Dermal), and click the *OK* button, or press *ENTER*.

D. Reading a New Assay

1. From the *File* menu, choose *New.*
2. Select *Assay,* and click *OK* button or press *ENTER*.
3. Select the desired protocol, and click the *OK* button or press *ENTER*.
4. Select the desired plate layout, and click the *OK* button, or press *ENTER*.
5. A series of screens will be displayed. You will need to enter the appropriate assay, and sample information. When you have finished, click the *OK* button.
   Note: The *Sample Number* for each sample must be entered. The *Sample Number* is used as the filename for the data file that will contain the results of the assay.
6. The plate reader will initialize. Load the assay plate, and click on the *Continue* button or press *ENTER*.
7. After the data collection has been completed, a dialog box will be displayed indicating if the assay was qualified or unqualified. Press the *OK* button to continue. If an "Inhibition Check" prompt does not appear, proceed to Step E to print the processed report.

E. Performing an Inhibition Check

1. If an inhibition check is required, a series of screens will appear indicating the specific well(s) on the assay plate that require an inhibition check.
2. Add 25 µl of the inhibition check to the appropriate well(s).
3. Wait 5 minutes, load the plate, then click the *Continue* button.
4. A dialog box will indicate if the assay was qualified or unqualified. Remove the plate, and press the *OK* button to continue.

F. Performing an Inhibition Check

1. A window, which displays the processed assay section of the report, for **each** sample will be displayed. To open additional windows (such as the protocol, plate layout, calibration curve, response curve or plate data), go to the *View* menu, and choose the desired window to be viewed.
2. To print the complete processed report, go to the *File* menu, and click the *Print* button. (The defaults have already been selected for you.)
3. To print additional sections of the report, select the section, and click on the *Print* button.

IRRITECTION® SOFTWARE FOR WINDOWS™
DETAILED INSTRUCTIONS

SECTION ONE: SETTING UP

I. Introduction:

Thank you for purchasing the Irritection® Software, a program designed to make your assay processing easier and more productive.

Prior to using the Irritection Software, make sure your package contains the following items:

1. InVitro International License Agreement (to be read carefully before proceeding).
2. Irritection software User's Manual for Windows™.
3. Irritection Software Diskette.

II. Installing the Software Program at a Working Station:

1. It is recommended that you make a backup copy of the Irritection Software diskette using the DOS Diskcopy command. See your DOS manual for more information.
2. Insert the backup copy of the software disk created in Step 1 into the 3.5" disk drive.
3. From the *Program Manager,* go to the *File* menu, and select *Run.*
4. A Run dialog box will be displayed. Click on *Browse.*
5. From the Drives box, click the 3.5" disk drive (*a* or *b*). From the File Name Box, click *Setup.exe,* then click *OK*.
6. A Run dialog box will be displayed. Click *OK.*
7. The Irritection Software Setup will begin initialization. Click *Continue.*
8. A Destination Path dialog box will be displayed. Click *Continue.*
9. After the Software has been installed, a message will appearing stating, "Setup Succeeded." Click *OK.*

Note: If the installation was not successful, or if you would like to install the software on a network, please call your Technical Representative for assistance.

III. Setting Up Your Plate Reader:

1. Double-click on the Irritection Software icon to launch the program.

2. From the *Instrument* menu, choose *Setup.*
   You will need to enter the following system parameters (this needs to only be done once):

   Comm Port:
   Select the appropriate Comm Port.

   Filters (nm):
   The wavelength of each filter installed on the filter wheel of the plate reader will need to be entered in order for the program to choose the correct filter when reading. Enter zero to indicate an empty position.

   Serial Number:
   The serial number of the plate reader will need to be entered.

   Note: If you have the Cambridge plate reader, the serial number can be found inside the plate reader on the left corner panel. For the Molecular Devices or Dynatech plate reader, the serial number can be found on the rear panel.

   Instrument Type:
   Click the arrow in the Instrument Type box, and select the appropriate instrument type.

   Mix Time:
   Enter the desired seconds.

   Manual Mode:
   If enabled, user must manually enter all assay results.

   Air Blank:
   If enabled, all well reading will be zeroed against the air background.

   Note: The *Baud Rate, Data Bits, Parity,* and *Stop Bits* will correctly default for each instrument type. The Plate Type will default on Generic 96-Well.

3. When you finish, click *OK.*

SECTION TWO: READING NEW ASSAY

I. Selecting the Assay Method

1. From the *Method* menu, choose *Select.*
2. Select the desired assay method (Ocular, High Sensitivity Ocular, Dermal, or High Sensitivity Dermal).

II. Reading the Assay

1. From the *File* menu, choose *New.*
2. Select *Assay.*
3. Select the desired protocol.
4. Select the desired plate layout.
5. A series of screens will be displayed. You will need to enter the appropriate assay, and sample information.

   Note: The *Sample Number* for each sample must be entered. The *Sample Number* is used as a filename for the datafile that will contain the results of the assay.

6. The plate reader will initialize. Load the assay plate, and click on *Continue,* or press *ENTER.*

III. Checking Qualifications

1. After the data collection has been completed, a dialog box will be displayed indicating if the assay was qualified or unqualified if an inhibition check is not required.
2. In an inhibition check is required, a series of screens will appear indicating the specific well(s) on the assay plate that require an inhibition check.
3. Add 25 µl of inhibition check to appropriate well(s).
4. Wait 5 minutes, load the plate, then click *Continue.*
5. A dialog box will indicate if the assay was qualified or unqualified. Click *OK* to continue.

IV. Viewing/Printing Assay Results

1. A window, which displays the qualified/unqualified assay results section of the report, for each sample will be presented. To open additional windows of the sample file (such as the protocol, plate layout, calibration curve, response curve, or plate data) go the *View* menu, and choose the desired window to be viewed. To view other samples, activate the sample file by clicking on the sample file window.
   Note: For more information on how to use the View command, go to Section 5: Using the View Menu.

2. To print a complete processed report, go to the *File* menu, and click *Print.* (The defaults have already been selected for you.)

SECTION THREE: USING THE FILE MENU

I. New Command

A. Assay:

The New Assay command allows automatic reading to be performed by a plate reader or results to be entered manually by a user. To enable manual mode, go the *Instrument* menu, and choose *Setup*. Click on Manual Mode.

1. From the *File* menu, choose *New.*
2. Select *Assay,* and click *OK,* or press *ENTER.*
3. Select the desired protocol, and click *OK,* or press *ENTER.*
4. Select the desired plate layout, and *OK,* or press *ENTER*
5. A series of screens (denoted by underlined headings) will be displayed. The following assay and sample information will need to be entered:

ASSAY INFORMATION

Assay

Incubation Time:

Refer to your Irritection Assay System protocol to determine the appropriate incubation time (24 hours or 5 hours).

Technician Name:

Enter the user's name.

Quality Controls

The Quality Controls are automatically selected by the software.

Reagent

Kit Lot Number:

This number can be found on either the Range Specification Sheet (included with the kit), kit box or on the bottles.

Temperature:

Enter the Reagent temperature.

pH Before Activation:

Enter the pH value.

pH After Activation:

Enter the pH Value.

SAMPLE INFORMATION

Sample

Description:
Enter the sample's name or description.

Product Type:
Enter the sample's product class (e.g., cosmetic, surfactant, industrial chemical)

pH and concentration:
Enter the pH value and concentration.

Sample Number:
Select a sample number that will uniquely identify the sample about to be processed. This number will be used as the filename for the datafile that will contain the results of this assay.

Company:
Enter your company name.

Comments:
Additional information about this sample can be entered in this section.

Doses/Concentrations
Default values will appear in this section. To edit this section, enter the desired values for each dose/concentration, then select the appropriate measurement.

Note: The samples in the same assay can be a mixture of solids and liquids. If the plate reader fails to initialize, you will need to start and repeat steps 1-4 and edit the desired doses/concentrations.

Sample Other Results
Other Results:
If you have "other results" for a sample, enter those values in this section. Under Title, enter the test method; under Measure, enter the filter wavelength; under Units, enter the type of score scale (i.e., IDE, HIE, PDII); under the Score, enter the numerical irritation score.

6. Click *OK.*

B. Protocol:

The New Protocol command allows the creation of a new protocol file (.rpr).

1. From the *File* menu, choose *New.*
2. Select *Protocol,* and click *OK,* or press *ENTER.*
3. A dialog box will be displayed asking if you would like to base the "new protocol" on an existing protocol. If you click *Yes*, and existing list of protocols will be displayed. Select the desired existing protocol.
4. A series of screens (denoted by underlined headings) will be displayed. The following protocol information will need to be entered:

PROTOCOL INFORMATION

Protocol:
Enter the name of the protocol you are creating.

Technician:
Enter the name of the user.

Score Precision:
The score precision specifies the number of digits (1 or 2) to be given after the decimal point for the irritancy scores. Enter "1" for the Ocular Assay and "2" for the Dermal, and High Sensitivity Ocular and Dermal Assays.

Filter Wavelength:
Enter the appropriate wavelength (400 nm for the Ocular and High Sensitivity Ocular Assays and 470 nm for the Dermal and High Sensitivity Dermal Assays).

Number of History Entries Required for Calibrator Substitution from History:
Enter the number of history entries required in order for a calibrator substitution from the history to be performed. (This is only applicable if the Standard Deviation or Percentage Test has been enabled.)

History Recording Enabled:
Enables each calibrator and quality control value to be recorded for every assay evaluated with this protocol. The recorded values are used for calculations for the standard deviation and percentage tests. Additionally, the history is used for the calculation of substitution values for unqualified calibrators.

Allow Calibrator Substitution from History:
Allows unqualified calibrators to be replaced by a "qualified substitute value" (running average of all history entries for that calibrator) generated from the history of the assay.

Automatic Setting of Assay Date:
Enables the assay date to be automatically recorded on the software printouts.

Automatic Graph Scaling of Score:
Enables the software to scale graphs based on the highest reading obtained.

Upper Score (if not using Automatic Graph Scaling for Score):
Enter the desired upper score.

Assay Method
The Assay Method is automatically selected by the software.

Doses/Concentrations
Enter the appropriate doses/concentrations.

Note: This section is only used if manual data entry has been enables. Default values for automatic reading by the plate reader are stored in the plate layout files (.rpt).

CALIBRATOR DEFINITIONS
Most of this information can be found on the range specification data sheet provided with the kit. Enter Cal 0 through Cal 3 under the Label heading. Enter the Calibrator 0 through Calibrator 3 under the Name heading. Enter the "Score", which is the known *in vivo* value for each calibrator. Enter the "OD" value, which is the mean OD value derived from the ranges established from each lot. Enter the "Low and High" values, which are derived from the ranges established from each lot.

QUALITY CONTROL DEFINITIONS
This information can be found on the range specification sheet provided with the kit. Enter QC 1 and QC 2 under the Label heading. Enter Quality Control 1 and Quality Control 2 under the Name heading. Enter the "Score", which is the mean score derived from the ranges established from each lot. Enter the "Low and High" values, which are derived from the ranges established from each lot.

Note: The Quality Controls included with the Irritection Assay system do not require blank readings.

Number of Quality Controls Used in Assay:
Enter the number of Quality Controls used.

Irritection Class Definitions:
Enter the Irritection class name, and the lower and upper score range limits.

Allow 10% borderline classifications:
Allows a sample that is within +/- 10% of a class boundary to be assigned an irritation class that is composed of the class below and above the boundary.

CALIBRATOR AND QUALITY CONTROL QUALIFICATION TESTS
Qualification Enabled:
If enabled, one of the following three testes will need to be selected for assay qualification: (1) Range Test, (2) Standard Deviation Test, or (3) Percentage Test.
If calibrator and quality control qualification is not enabled in the associated protocol, then qualification ranges are not computed and calibrator and quality control qualification codes will indicate that qualification has been bypassed.

Range Test:
If the "Range Test" is selected, qualification will be based on the calibrator/quality control upper and lower OD/scores limits specified in the protocol (i.e., if the OD/score is within the specified range, the calibrator/quality control will qualify.)

Standard Deviation Test:
If the "Standard Deviation Test" is selected, and the assay history and required number of assay history entries are available, then the lower and upper limits for the calibrator ODs and quality control scores are calculated from the history using the running average and standard deviation of the calibrator/quality control. If the assay history is not available or there are insufficient assay history entries, a dialog box will be displayed stating that the qualification test will be changed to the "Range Test".

Percentage Test:
If the "Percentage Test" us selected, and the assay history and required number of assay history entries are available, then the lower and upper limits for the calibrator/quality control OD/score will be calculated using the running average obtained from the assay history and the percentage tolerance specified in the protocol. If the assay history is unavailable or there are insufficient assay history entries, then the lower and upper limits for the calibrator/quality control OD/scores will be calculated using the calibrator/quality control OD/scores and percentage tolerance specified in the associated protocol.

SD Tolerance (+/- SD):

Value entered is used to establish a range and specifies the standard error of a calibrator/quality control. (e.g., mean ± 2D)

% Tolerance (+/- %):
Value entered is used to establish a range and specifies the percent error of a calibrator/quality control.

Number of History Entries Required for SD or % Testing:
Enter the number of entries required.

Maximum Number of Unqualified Quality Controls or Calibrators:
Specifies the maximum number of unqualified quality controls or calibrators an assay can have and still quality.

CONCENTRATION RESPONSE CURVE QUALIFICATION TESTS
Concentration Response Curve Qualification Enabled:
Enables qualification of a concentration curve by limits or curve-fitting method.

Concentration Above the Highest Qualified Concentration Fail:
Enables concentration above the highest qualified concentration to fail.

Include Unqualified Concentrations on Concentration Response Curve:
Includes unqualified concentrations on response curve.

Methods Used to Find the Highest Qualified Concentration
Limits Method:
Limits Method will qualify an increasing or flat curve. Each concentration is tested beginning with the highest concentration, and progressing to the lowest concentration until a concentration fails a test. Each concentration is tested by pairing it with every qualified concentration below itself and performing one of three tests (Low Range Limit, Mid Range Limit, High Range Limit). The test used on these individual pairings depends on the net OD values of the concentration under test, as compared to the low and mid range limits specified in the protocol. If the net OD is less than the low range limit, the low range limit test is used. If the net OD is less than the mid range limit test, then the mid range is used. Otherwise, the high range limit test is used.

Curve Fitting Method:
The Curve Fitting method allows the user to interactively exclude any concentrations which are adversely affecting the curve fitting process. Manual exclusion of a concentration by the user only affects the values used to perform the

curve fitting. (To manually exclude a concentration, go to Section Four: Edit Menu). A second order polynomial is fitted to those qualified concentrations and associated net OD values which have not been manually excluded. If the curve is concave down and the peak is within the range of concentration levels tested, all concentrations will qualify. If the curve does not concave down or the peak is not within the range of concentration level specified in the assay then the software defaults to the Limits Method and will qualify an increasing or flat curve.

Limits Method

Upper Limit of Low Range:
The Low Range Limit Test will be performed on samples with the net ODs that are below this limit.

Upper Limit of Mid Range:
The Mid Range Limit Test will be performed on samples with net ODs that are below this limit.

Low Range Variability:
Qualification will be based on the net OD variability allowed between two consecutive concentrations when the Low Range Limit Test is performed.

Mid Range Variability:
Qualification will be based on the net OD variability allowed between two consecutive concentrations when the Mid Range Limit Test is performed.

DOSE RESPONSE CURVE QUALIFICATION TESTS

Dose Response Curve Qualification Enabled:
Enables qualification of a dose curve by the Empirical or Curve Fitting method.

Consecutive Qualified Doses Required:
Qualification requires consecutive qualified doses.

Doses Higher than any Failed Dose Also Fail:
Enables doses higher than any failed doses to also fail.

Require Highest Dose to Qualify:
Qualification requires highest dose to qualify.

Doses with Increasing Net ODs Qualify:
Qualification will include doses with increasing net ODs.

Doses Above the Highest Qualified Dose Fail:
Qualification will not include doses above highest qualified dose.

Include Unqualified Doses on Dose Response Curve:
Dose response curve will include unqualified doses.

Methods Used to Find the Highest Qualified Dose
Empirical Method:
The Empirical method will qualify an increasing or flat curve. The sample doses are tested in groups of three (e.g., doses 1 through 3, doses 2 through 4). The qualification depends on the net OD of the doses within a group. If any of the net ODs fall into the low, mid or high range, the Low Range Limit, Mid Range Limit, or High Range Limit Test, respectively, will be performed.

Curve Fitting Method:
The Curve Fitting method allows the user to interactively exclude any doses which are adversely affecting the curve fitting process. Manual exclusion of a dose by the user only affects the values used to perform the curve fitting. (To manually exclude a dose, go to Section Four: Edit Menu). A second order polynomial is fitted to those qualified doses and associated net OD values which have not been manually excluded or automatically eliminated. If the curve is concave down and the peak is within the range of dose levels used in the assay, then all of the doses of the curve are qualified. If the curve does not concave down or the peak is not within the range of dose levels specified in the assay, then the software defaults to the Empirical Method and will qualify an increasing or flat curve.

Minimum Number of Qualified Doses:
Qualification based on this minimum number of qualified doses.

Low Range Flatness
For OD <:
The Low Range Test will be performed when a sample net OD is below this value.

Pt to Pt <:
Qualification will be based on the net OD variability allowed between two consecutive doses.

Overall <:
Qualification will be based on the net OD variability allowed between the highest and lowest doses in a group of three doses.

Mid Range Flatness
For OD <:
The Mid-Range Test will be performed when a sample net OD is below this value.

Pt to Pt <:
Qualification will be based on the net OD variability allowed between two consecutive doses.

Overall <:
Qualification will be based on the net OD variability allowed between the highest and lowest doses in a group of three doses.

OTHER QUALIFICATION TESTS
Inhibition Test
None:
If enabled, an inhibition test will not be performed.

Highest:
An inhibition check will need to be performed if the net OD for the maximum qualified sample dose or concentration is < Cal 2.

Individual:
An inhibition check will need to be performed if the net OD for any selected sample dose or concentration is < Cal 2.

Individual Inhibition Check
Select the desired doses that require an inhibition check.

B0 Test
Minimum B0 OD:
Qualification is based on this minimum OD value.

Maximum B0 OD:
Qualification is based on this maximum OD value.

Blank Tests
Blank Qualification Enabled:
Enables each blank OD for each sample to be tested. The blanks must pass a range test, and in some cases, a blank flatness check.

Minimum Blank OD:
Qualification will be based on this minimum OD value.

Maximum Blank OD:
Qualification will be based on this maximum OD value.

Blank to Blank Flatness:
Qualification will be based on the blank OD variability allowed between tow consecutive doses.

Blank Overall Flatness:
Qualification will be based on the blank OD variability allowed between the highest and lowest doses in a group of three doses.

Net OD Tests
Net Qualification Enabled:
Enables each sample dose or concentration which has not failed a previous qualification test to be tested for net OD acceptability. The net OD must be within the limits specified in the protocol.

Minimum Net OD:
Qualification is based on this minimum OD value.

5. When you finished, click *OK.*

C. Plate Layout:

The New Plate Layout allows the creation of a new plate layout files (.rpt).

1. From the *File* menu, select *New.*
2. Select *Plate Layout,* and click *OK,* or press *ENTER.*
3. A dialog box will be displayed asking if you would like to base the "new plate layout" on an existing plate layout. If you click *Yes*, and existing list of plate layouts will be displayed. Select the desired existing plate layout.
4. A screen will appear and the appropriate plate layout information will need to be entered. The dose/concentration settings entered will be default values that appear on the sample information screen when automatically reading an assay. When you have finished, click *OK* or press *ENTER.*
5. A plate layout will be displayed. With your mouse "drag" the labeled wells into the desired plate configuration. Use the "empty well" to correct any mistakes.
6. When you finish, go to the *File* menu, and select *Close.*

7. A dialog box will appear asking if you would like to save the new plate layout. Select *Yes.*

II. Open Command

1. From the *File* menu, select *Open.*
2. In the File Name box, type or select the name of the document you want to open.
   Note: As you select the file you want, the protocol and plate layout names will appear in the boxes below. If you do not see the name of the file you want, select a different type of file from the Lit of Files of Type box (sample files=.rpk; protocol file=.rpr; plate layout file=.rpt; history file=.rpd).
3. When you finish, select *OK.*

III. Close Command

1. From the *File* menu, select *Close.* This will close the active window.
2. If a document has changes you have not saved, a dialog box will be displayed asking if you want to save the changes before closing. If you choose Yes, but have not given the file name, the Save As dialog box will be displayed.

IV. Save/Save As Commands

1. To save existing or open file(s), go to the *File* menu, and select *Save.*
2. To save a changed sample file name (number), go to the *File* menu, and select *Save As.* A Save As dialog box will be displayed. Type the new file name in the File Name box, and select *OK*.
   Note: When a new protocol or plate layout file is created, DOS file names are automatically assigned and saved with the file.

V. Print Command

1. To print, go to the *File* menu, and select *Print.*
2. The defaults for the Print Selection have already been set for you. To print additional sections, click on the box next to the desired selection. When you finish, select *OK.*

VI. Print Setup Command

1. From the File menu, select *Print Setup.*
2. Select the appropriate print setup options.
3. When you finish, select *OK.*

VII. Exit Command

1. To exit out of Irritection Software, go to the *File* menu, and select *Exit.*

SECTION FOUR: USING THE EDIT MENU

I. Assay Command

1. From the *File* menu, choose *Open.* Open the desired sample (.rpk) file.
2. From the *Edit* menu, choose *Assay.*
3. A series of screens will be displayed. Edit the assay report section of the sample file. When you finish, click *OK.*
4. A dialog box will be displayed stating if the "edited assay" was qualified or not qualified.

II. Protocol Command

Note: you can either edit the protocol section of a sample file (.rpk), or edit the protocol file (.rpr) itself. Changes made on the active file only be updated for that specific file (i.e., if a sample file (.rpk) is open and you edit the protocol section of that sample file, the protocol for that sample file will be update, but the protocol file (.rpr) will not be update.)

1. From the *File* menu, choose *Open.* Open either the desired sample (.rpk) file or protocol (.rpr) file.
2. From the *Edit* menu, choose *Protocol.*
3. A series of screens will be displayed. Edit the desired sections. When you finish, click *OK.*

   Note: if you choose to edit the protocol section of a sample file, you will not see the edited protocol changes unless you view the protocol report for that sample file. For more details on the View menu, go to Section Five: Using the View Menu.

4. After you have edited the protocol section of a sample file (.rpk), you will need to recompute for assay qualification. From the *Edit* menu, choose *Recompute Assay.*
5. A dialog box will be displayed stating if the assay has qualified using the "edited protocol".
6. To save the changed, go to the *File* menu, choose *Save.*

III. Plate Layout Info. Command

Note: you can either edit the plate layout info. Section of a sample file (.rpk), or edit the plate layout file (.rpt) itself. Changes made on the active file will only be updated for that specific file.

1. From the *File* menu, choose *Open.* Open either the desired sample (.rpk) file or plate layout (.rpt) file.
2. From the *Edit* menu, choose *Plate Layout Info.*
3. Edit the sections of the Plate Layout Info. When you finish, click *OK.*

IV. Scales Command

Note: You can edit the scales of either your sample calibrator or sample response curve.

1. From the *File* menu, choose *Open.* Open the sample (.rpk) you wish to edit.
2. From the *View* menu, choose *Calibrator* or *Response Curve.*
3. From the *Edit* menu, choose *Scales.*
4. Edit the X and/or Y axis maximum values. When you finish click *OK.*

V. Response Curve Command

Note: You can only edit the response curve if "curve fitting" was selected in the associated protocol as the method used to qualify the response curve.

1. From the *File* menu, choose *Open.* Open the sample (.rpk) you wish to edit.
2. From the *View* menu, choose *Response Curve.*
3. From the *Edit* menu, choose *Response Curve.*
4. Exclude the desired concentration(s)/dose(s). When you finish click *OK.*

VI. Recompute Assay Command

Note: If the protocol section of a sample file has been edited, qualification checks based on the edited protocol must be performed.

1. From the *Edit* menu, choose *Recompute Assay.*
2. Qualification checks will be performed based on the edited protocol.
3. A dialog box will appear stating whether the assay was qualified or unqualified.

VII. History Command

A. Edit History Entries

1. From the *Edit* menu, choose *History.*
2. Select *Edit History Entries.*
3. Select the history you wish to edit by protocol name. When you finish, click *OK.*
4. A series of screens from Cal 0 to QC 2 will be displayed. Edit the desired sections by either clicking *Insert* or *Delete.* When you finish, click *OK.*

B. Update History with all Sample Files/Rebuild History with all Sample Files

1. From the *Edit* menu, choose *History.*
2. Select *Update History with all Sample Files/Rebuild History with all Sample Files.* When you finish, Click *OK.*

C. Clear History

1. From the *Edit* menu, choose *History.*
2. Select *Clear History.* When you finish, click *OK.*

D. Recover Previous History

1. From the *Edit* menu, choose *History.*
2. Select *Recover Previous History.* When finish, click *OK.*

SECTION FIVE: USING THE VIEW MENU

I. View Command

Note: The View Command only works with an active sample file (.rpk)

1. From the *File* menu, choose *Open.* Open the desired sample file.
2. From the *View* menu, choose either Assay, Protocol, Plate Layout, Calibrator Curve, Response Curve or Plate Data.

SECTION SIX: USING THE INSTRUMENT MENU

I. Retract/Extend Plate Commands

Note: The retract and extract commands allows you to manually retract or extend the plate reader.

1. To retract/extend the plate from the plate reader, go to the *Instrument* menu, and choose *Retract/Extract.*

II. Setup

The following steps should be performed during the software installation process.

1. From the *Instrument* menu, and choose *Setup.*
2. You will need to enter the following system parameters (this needs to only be done once):

   Comm Port:
   Select the appropriate Comm Port.

Filters (nm):
The wavelength of each filter installed on the filter wheel of the plate reader will need to be entered in order for the program to choose the correct filter when reading. Enter zero to indicate an empty position.

Serial Number:
The serial number of the plate reader will need to be entered.
Note: If you have the Cambridge plate reader, the serial number can be found inside the plate reader on the left corner panel. For the Molecular Devices or Dynatech plate reader, the serial number can be found on the rear panel.

Instrument Type:
Click the arrow in the Instrument Type box, and select the appropriate instrument type.
Note: The *Baud Rage, Data Bits, Parity,* and *Stop Bits* will correctly default for each instrument type. The Plate Type will default on Generic 96-Well.

Mix Time:
Enter the desired seconds.

Manual Mode:
If enabled, user must manually enter all assay results.

Air Blank:
If enabled, the B0 well value will not be subtracted from every well of the reading plate.

SECTION SEVEN: USING THE METHOD MENU

I. Select Command

1. From the *Method* menu, and choose *Select.*
2. Select the desired *Assay Method.*
   Note: The data path displays the directory where the sample, protocol, plate layout and history files are stored.

SECTION EIGHT: WINDOW MENU

Note: The window menu allows you to arrange your active files in either cascade, tile horizontally, or tile vertically. All active window files are listed under the window.

Irritection® Assay System

Instruction Manual

Chapter 8
Installation Information

InVitro International
17751 Sky Park East, Suite G
Irvine, California, 92614

TRAINING SCHEDULE:
OCULAR AND DERMAL RESPONSE ASSAYS[1]

DAY 1:

8:00–8:30 AM INTRODUCTION TO THE OCULAR RESPONSE ASSAYS

- Description of Background and Scientific Basis
- Comparison of Irritection Assay System and *In Vivo* Test Results
- Description of Kit components
- Overview of Protocols and Procedures

8:30–10:00 AM PERFORM A 5 HOUR OCULAR IRRITECTION ASSAY

- Utilize the Surfactant Protocol
- Perform a Concentration-Dependent Study (4 Samples with 5 Concentrations)

10:00–10:30 AM INTRODUCTION TO THE DERMAL RESPONSE ASSAYS

- Description of Background and Scientific Basis
- Comparison of Irritection Assay System and *In Vivo* Test Results
- Description of Kit components
- Overview of Protocols and Procedures

10:30-12:00 PM PERFORM A 24 HOUR DERMAL IRRITECTION ASSAY

- Utilize the Cosmetic Protocol
- Perform a Volume-Dependent Study (4 Samples with 5 Volumes)

12:00-1:00 PM BREAK FOR LUNCH

1:00-2:30 PM IRRITECTION SOFTWARE TRAINING

- Install Irritection software for Windows Version 1.00
- Description of the Software and its use

[1] Prior to training, please review all protocol procedures, prepare samples as required, and adjust the incubator to maintain a temperature of 25˚C.

2:30–3:30 PM COMPLETE THE OCULAR RESPONSE ASSAY

- Read the Assay Results
- Analyze and Interpret the Data

DAY 2:

9:30–11:30 AM REVIEW AND DISCUSSION

- Review Ocular Response Assay Results
- Discuss Qualification of Assay Results
- Discuss Data Interpretation
- Describe Troubleshooting Techniques

11:30-12:30 PM COMPLETE THE DERMAL RESPONSE ASSAY

- Read the Assay Results
- Analyze and Interpret the Data

12:30-1:30 PM BREAK FOR LUNCH

1:30-2:30 pm FINAL QUESTIONS AND COMMENTS

CUSTOMER-SUPPLIED REQUIRED EQUIPMENT

96-Well microtiter platereader such as:

Cambridge (filters: 400 nm and 470 nm)

Molecular Devices (filters: 405 nm and 450 nm)

Dynatech (filters: 405 nm and 450 nm)
User of Dynatech platereader may require Smart Cable (SC817RM) from IQ Technology (1-800-227-2817)

IBM compatible PC with Irritection Software

Incubator maintained at 25˚C ± 1˚C.

Balance (110 g capacity)

pH Meter

Vortex mixer

Positive displacement micro pipetter, 5 – 250 µl capacity

Multichannel expandable pipetter, 15 – 1250 µl capacity*

Disposable reservoir trays, 100 ml capacity

Graduated cylinder, 100 ml capacity

Beaker, 100 ml capacity

Funnel, 12.5 cm diameter

Plastic forceps

Plastic wrap

* Option: 8 channel manual pipetter, 0 – 250 µl capacity and a repeating pipetter, 250 – 1250 µl capacity.

Irritection® Assay System

Instruction Manual

Chapter 9
Material Safety Data Sheets

InVitro International
17751 Sky Park East, Suite G
Irvine, California, 92614

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40000**

InVitro International
17751 Sky Park East, Ste. G
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Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Reagent Powder
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

To our knowledge, this product contains no hazardous components.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: White lyophilized powder.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Appropriate Foam.
Special Firefighting Procedure: Wear yourself contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion, or skin absorption. Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.
First Aid: If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
In case of skin contact, flush with copious amounts of water.
If inhaled, remove to fresh air. If breathing becomes difficult, give oxygen.

In case of contact with eyes, flush with copious amounts of water for at least 15 minutes.
Assure adequate flushing by separating the eyelids with fingers.
Call a physician.

Section VII: REACTIVITY DATA

Stability: Stable
Hazardous Combustion or Decomposition Product: The nature of decomposition products are not known.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wear safety goggles and gloves. Sweep up, place in a bag and hold for waste disposal. Avoid raising dust.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Mechanical exhaust required.
Label precautionary statements: Caution – the chemical, physical and toxicological properties of this product have not been thoroughly investigated. Exercise due care.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40001**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Hydrating Solution
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An inorganic aqueous solution, slightly acidic to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Flashpoint: NA
Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through skin.
First Aid: In case of eye contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing

becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Alkali Metals (e.g. Lithium and Sodium).
Hazardous Combustion or Decomposition Product: None.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40002**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Blanking Buffer
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A buffered solution, alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Dry Chemical Powder.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through skin.
First Aid: In case of eye contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong acids and strong bases.
Hazardous Combustion or Decomposition Product: Nature of decomposition products not known.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, skin, or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40003**

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Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Activator
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Inorganic aqueous solution, acidic to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder, Alcohol or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhaled, ingestion or skin absorption.
First Aid: In case of eye contact, immediately flush eyes with copious amount of water. In case of skin contact, immediately wash skin with soap and copious amounts of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong bases, strong oxidizing agents, air sensitive, moisture sensitive.
Hazardous Combustion or Decomposition Product: Sulfur Oxides.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing.

Section X: SPECIAL PRECAUTIONS

Avoid contact with eyes, skin, or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Irritant. Store in a cool dry place.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40004**

InVitro International
17751 Sky Park East, Ste. G
Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Inhibition check
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A dilute aqueous solution of a weak organic base, slightly alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Sprays.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Anionic detergents and nitrates.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide, Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, skin, or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40005**

InVitro International
17751 Sky Park East, Ste. G
Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Cal 0
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A buffered solution of a pH balanced inorganic base.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Sprays.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles. Strong acids or bases.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin, or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40006**

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Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Cal 1
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Solution of weak organic acid and poly-ol alcohol

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless, viscous liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Flash Point: NA
Extinguishing Media: Water Spray , Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through the skin. Causes eye and skin irritation.
First Aid: In case of contact, flush eyes or skin with copious amount of water.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Oxidizing Agents, Bases, Reducing Agents.
Hazardous Combustion or Decomposition Product: Toxic Fumes of Carbon Monoxide and Carbon Dioxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40007**

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Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Cal 2
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Solution of weak organic acid and poly -ol alcohol

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless, viscous liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Flash Point: NA
Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through the skin. Causes eye and skin irritation. Material is irritating to mucous membranes and upper respiratory tract.

First Aid: In case of contact, immediately flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Oxidizing Agents, Bases, Reducing Agents.
Hazardous Combustion or Decomposition Product: Toxic Fumes of Carbon Monoxide and Carbon Dioxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40008**

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Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response Cal 3
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Weak aqueous solution of an organic acid.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed or absorbed through the skin.
First Aid: In case of contact, immediately flush eyes or skin with copious amount of water. Remove contaminated clothing and shoes. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Bases.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Do not breathe vapor. Readily absorbed through skin. Wash thoroughly after handling.

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MATERIAL SAFETY DATA SHEET **40009**

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Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response QC 1
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous solution of inorganic salt.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if inhaled or swallowed. May cause eye and skin irritation. To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.
First Aid: In case of contact, immediately flush eyes or skin with copious amount of water.
In case of contact, immediately wash the skin with soap and copious amounts of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing

becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Alkali Metals (e.g. Lithium, Sodium)
Hazardous Combustion or Decomposition Product: None.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear safety goggles, chemical-resistant gloves, and other protective clothing. Safety shower and eye bath. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed.

Section X: SPECIAL PRECAUTIONS

Store in a cool, dry place.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40010**

InVitro International
17751 Sky Park East, Ste. G
Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Ocular Response QC 2
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Weak aqueous solution of an organic acid.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed or absorbed through skin.
First Aid: In case of contact, immediately flush eyes or skin with copious amount of water. Remove contaminated clothing and shoes. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Bases.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Do not breathe vapor. Readily absorbed through skin. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40011**

InVitro International
17751 Sky Park East, Ste. G
Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response
Reagent Powder

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

To our knowledge, this product contains no hazardous components.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Light Yellow lyophilized powder.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion, or skin absorption. Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.
First Aid: If swallowed, wash out mouth with water, provided person is conscious.
In case of skin contact, flush with copious amount of water.

If inhaled, remove to fresh air. If breathing becomes difficult, give oxygen.
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers.
Call a physician.

Section VII: REACTIVITY DATA

Stability: Stable.
Hazardous Combustion or Decomposition Product: The nature of decomposition products are not known.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wear safety goggles and gloves. Sweep up, place in a bag and hold for waste disposal. Avoid raising dust.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Mechanical exhaust required. Label Precautionary Statements: Caution – The chemical, physical and toxicological properties of this product have not been thoroughly investigated. Exercise due care.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40012**

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Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response
Hydrating Solution

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An inorganic aqueous solution, slightly acidic to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Flashpoint: NA
Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through skin.

Causes eye and skin irritation.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Alkali Metals (e.g. Lithium and Sodium).
Hazardous Combustion or Decomposition Product: None.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40013**

InVitro International
17751 Sky Park East, Ste. G
Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response
Blanking Buffer

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A buffered solution, alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Dry Chemical Powder.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through skin. Causes eye and skin irritation.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing

becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong acids and strong bases.
Hazardous Combustion or Decomposition Product: Nature of decomposition products not known.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40014**

InVitro International
17751 Sky Park East, Ste. G
Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response - **Cal 0**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A buffered solution of a pH balanced inorganic base.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Spray.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong acids or bases.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40015**

InVitro International
17751 Sky Park East, Ste. G
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Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response – **Cal 1**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous, inorganic acid solution.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless, odorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosions Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if inhaled or swallowed. May be harmful if absorbed through the skin. Causes severe eye irritation. May cause skin irritation.
First Aid: In case of contact, immediately flush eyes or skin with copious amount of water. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Bases.
Hazardous Combustion or Decomposition Product: Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid inhalation. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Keep tightly closed.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40016**

InVitro International
17751 Sky Park East, Ste. G
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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response – **Cal 2**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous, inorganic acid solution.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if inhaled or swallowed. May be harmful if absorbed through skin. Causes severe eye irritation. May cause skin irritation.
First Aid: In case of contact, flush eyes or skin with copious amount of water. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Bases.
Hazardous Combustion or Decomposition Product: Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid inhalation. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40017**

InVitro International
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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response – **Cal 3**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous, inorganic acid solution.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if inhaled or swallowed. May be harmful if absorbed through the skin. Causes severe eye irritation. May cause skin irritation.
First Aid: In case of contact, flush eyes or skin with copious amount of water. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Bases.
Hazardous Combustion or Decomposition Product: Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid inhalation. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40018**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response – **QC 1**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous solution of inorganic salt.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion or skin absorption. May cause irritation. To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly.
First Aid: In case of contact, immediately flush eyes with copious amount of water.
In case of contact, immediately wash skin with soap and copious amount of water.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Alkali Metals (e.g. Lithium, Sodium).
Hazardous Combustion or Decomposition Product: None.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Do not breath vapor. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40019**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response – **QC 2**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An diluted aqueous solution of a weak organic base, slightly alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Spray.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Anionic agents and nitrates.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide, Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40020**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response Activator

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Inorganic aqueous solution, acidic to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder, Alcohol or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosions Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion or skin absorption.
First Aid: In case of eye contact, immediately flush eyes with copious amount of water. In case of skin contact, immediately wash skin with soap and copious amounts of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing

becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong bases, strong oxidizing agents, air sensitive, moisture sensitive.
Hazardous Combustion or Decomposition Product: Sulfur Oxides.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin an clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Irritant. Store in a cool dry place.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **40021**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Ocular Response Inhibition Check

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A diluted aqueous solution of a weak organic base, slightly alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder, or Water Spray.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Anionic agents and nitrates.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide, Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50000**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response Reagent Powder

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

To out knowledge, this product contains no hazardous components.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: White lyophilized powder.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Appropriate Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion, or skin absorption. Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.
First Aid: If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
In case of skin contact, flush with copious amount of water.

If inhaled, remove to fresh air. If breathing becomes difficult, give oxygen.
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers.
Call a physician.

Section VII: REACTIVITY DATA

Stability: Stable.
Hazardous Combustion or Decomposition Product: The nature of decomposition products are not known.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wear safety goggles and gloves. Sweep up, place in a bag and hold for waste disposal. Avoid raising dust.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Mechanical exhaust required.
Label precautionary statements: Caution – The chemical, physical and toxicological properties of this product have not been thoroughly investigated. Exercise due care.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50001**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response Hydrating Solution

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An inorganic aqueous solution, alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Flashpoint: NA
Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder, or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through the skin. Causes eye and skin irritation.

First Aid: In case of contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Alkali Metals (e.g. Lithium, Sodium) and Acids.
Hazardous Combustion or Decomposition Product: None.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50002**

InVitro International
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Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response Blanking Buffer

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A buffered solution, alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Dry Chemical Powder
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong acids and strong bases.
Hazardous Combustion or Decomposition Product: Nature of decomposition products not known.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50003**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response Activator

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Inorganic aqueous solution, acidic to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Regular Foam
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosions Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhaled, ingestion or skin absorption. Causes eye and skin irritation.
First Aid: In case of eye contact, immediately flush eyes with copious amount of water. In case of skin contact, immediately wash skin with soap and copious amounts of water.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong bases, strong oxidizing agents, air sensitive, moisture sensitive.
Hazardous Combustion or Decomposition Product: Sulfur Oxides.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing.

Section X: SPECIAL PRECAUTIONS

Avoid contact with eyes, skin and clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Irritant. Store in a cool dry place.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50004**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response Inhibition Check

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A diluted aqueous solution of a weak organic base, slightly alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Spray
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Anionic agents and nitrates.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide, Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50005**

InVitro International
17751 Sky Park East, Ste. G
Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response – **Cal 0**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous organic salt solution, alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Regular Foam

Section VI: HEALTH HAZARD DATA

Acute Effects: Direct contact may cause eye irritation. Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water. If irritation persists, call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong Oxidizing Agents.

Hazardous Combustion or Decomposition Product: Toxic fumes of Carbon Dioxide and Carbon Monoxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.

Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50006**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response – **Cal 1**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A diluted aqueous solution of a weak organic base, slightly alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Spray.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Anionic agents and nitrates.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide, Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50007**

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Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response – **Cal 2**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A diluted aqueous solution of a weak organic base, slightly alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Spray.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Anionic agents and nitrates.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide, Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50008**

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Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response – **Cal 3**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Solution of a weak organic acid and poly –ol alcohol.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Flashpoint: NA
Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through the skin. Causes eye and skin irritation.

First Aid: In case of contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Oxidizing Agents, Bases, Reducing Agents.
Hazardous Combustion or Decomposition Product: Toxic Fumes of Carbon Monoxide and Carbon Dioxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed.

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MATERIAL SAFETY DATA SHEET **50009**

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Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response – **QC 1**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous solution of inorganic salt.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion or skin absorption. May cause irritation. To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.
First Aid: In case of contact, immediately flush eyes with copious amount of water.
In case of contact, immediately wash skin with soap and copious amount of water.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Alkali Metals (e.g. Lithium, Sodium).
Hazardous Combustion or Decomposition Product: None.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Do not breathe vapor. Wash thoroughly after handling. Keep tightly closed.

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MATERIAL SAFETY DATA SHEET **50010**

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Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: Dermal Response - **QC 2**
SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous, organic salt solution.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless, odorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosions Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if inhaled or swallowed. May be harmful if absorbed through skin. Causes severe eye irritation. May cause skin irritation.
First Aid: In case of contact, immediately flush eyes or skin with copious amount of water. Assure adequate flushing of the eyes by separating the eyelids with fingers.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing

becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: None.
Hazardous Combustion or Decomposition Product: Toxic Fumes of Carbon Monoxide, Carbon Dioxide and Sulfur Oxides.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid inhalation. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Irritant. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50011**

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Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response
Reagent Powder

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

To our knowledge, this product contains no hazardous components.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Light yellow lyophilized powder.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Appropriate Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion, or skin absorption. Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.
First Aid: If swallowed, wash out mouth with water, provided person is conscious.
In case of skin contact, flush with copious amount of water.

If inhaled, remove to fresh air. If breathing becomes difficult, give oxygen.
In case of contact with eyes, flush with copious amount of water for at least 15 minutes. Assure adequate flushing of the eyes by separating the eyelids with fingers.
Call a physician.

Section VII: REACTIVITY DATA

Stability: Stable.
Hazardous Combustion or Decomposition Product: The nature of decomposition products are not know.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wear safety goggles and gloves.
Sweep up, place in a bag and hold for waste disposal. Avoid raising dust.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Mechanical exhaust required.
Label precautionary statements: Caution – The chemical, physical and toxicological properties of this product have not been thoroughly investigated. Exercise due care.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50012**

InVitro International
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Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response
Hydrating Solution

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An inorganic aqueous solution, slightly acidic to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.
Boiling Point: NA
Vapor Pressure: NA

Section V: FIRE AND EXPLOSION HAZARD

Flashpoint: NA
Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through skin.

Causes eye and skin irritation.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Alkali Metals (e.g. Lithium and Sodium)
Hazardous Combustion or Decomposition Product: None.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50013**

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Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response
Blanking Buffer

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A buffered solution, alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Dry Chemical Powder.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed. May be harmful if inhaled or absorbed through skin. Causes eye and skin irritation.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes

difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong acids and strong bases.
Hazardous Combustion or Decomposition Product: Nature of decomposition products not known.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50014**

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Irvin, CA 92614
Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response – Cal 0

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A buffered solution of a pH balanced inorganic base.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Regular Foam.

Section VI: HEALTH HAZARD DATA

Acute Effects: Direct contact my causes eye irritation. Harmful if swallowed.
First Aid: In case of contact, flush eyes with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong acids and bases.
Hazardous Combustion or Decomposition Product: Toxic Fumes of Carbon Dioxide and Carbon Monoxide.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50015**

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Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response - **Cal 1**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous, inorganic acid solution.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless, odorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosions Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if inhaled or swallowed. May be harmful if absorbed through the skin. Causes severe eye irritation. May cause skin irritation.
First Aid: In case of contact, immediately flush eyes or skin with copious amount of water. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Bases.
Hazardous Combustion or Decomposition Product: Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid inhalation. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Keep tightly closed.

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MATERIAL SAFETY DATA SHEET **50016**

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Telephone: (714) 851-8356
Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response - **Cal 2**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous, inorganic acid solution.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless, odorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosions Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if inhaled or swallowed. May be harmful if absorbed through the skin. Causes severe eye irritation. May cause skin irritation.
First Aid: In case of contact, immediately flush eyes or skin with copious amount of water. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Bases.
Hazardous Combustion or Decomposition Product: Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid inhalation. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Keep tightly closed.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. In Vitro International shall not be held liable for any damage resulting from handling or from contact with the above product. The information contained herein is furnished without warranty of any kind and is intended for the use only by persons having adequate related technical skills and at their own discretion and risk. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50017**

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Fax: (714) 851-0563

FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response - **Cal 3**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous, inorganic acid solution.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosions Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if inhaled or swallowed. May be harmful if absorbed through the skin. Causes severe eye irritation. May cause skin irritation.
First Aid: In case of contact, immediately flush eyes or skin with copious amount of water. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
Wash contaminated clothing before reuse.

Section VII: REACTIVITY DATA

Incompatibles: Bases.
Hazardous Combustion or Decomposition Product: Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid inhalation. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50018**

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Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response - **QC 1**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

An aqueous solution of inorganic salt.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Water Spray, Carbon Dioxide, Dry Chemical Powder or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion or skin absorption. May cause irritation. To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.
First Aid: In case of contact, immediately flush eyes with copious amount of water.
In case of contact, immediately wash skin with soap and copious amount of water.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Alkali Metals (e.g. Lithium, Sodium).
Hazardous Combustion or Decomposition Product: None.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Do not breathe vapor. Wash thoroughly after handling. Keep tightly closed.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50019**

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FOR EMERGENCY INFORMATION CALL:
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Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response - **QC 2**

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A diluted aqueous solution of a weak organic base, slightly alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Spray.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious. Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Anionic agents and nitrates.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide, Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50020**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response Activator

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

Inorganic aqueous solution, acidic to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder, Alcohol or Regular Foam.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual Fire and Explosions Hazards: Emits toxic fumes under fire conditions.

Section VI: HEALTH HAZARD DATA

Acute Effects: May be harmful by inhalation, ingestion or skin absorption. Causes eye and skin irritation.
First Aid: In case of eye contact, immediately flush eyes with copious amount of water. In case of skin contact, immediately wash skin with soap and copious amount of water.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Strong bases, strong oxidizing agents, air sensitive, moisture sensitive.
Hazardous Combustion or Decomposition Product: Sulfur Oxides.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Avoid contact with eyes, skin and clothing. Avoid prolonged exposure. Wash thoroughly after handling. Irritant. Store in a cool dry place.

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Date: 08/96 Revision: 1

MATERIAL SAFETY DATA SHEET **50021**

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FOR EMERGENCY INFORMATION CALL:
1-800-535-5053

Section I: PRODUCT IDENTIFICATION

Product Name: High Sensitivity Dermal Response Inhibition Check

SPECIFIC CONTENTS ARE TRADE SECRET

Section II: HAZARDOUS COMPONENTS

A diluted aqueous solution of a weak organic base, slightly alkaline to litmus.

Section III: TOXICITY HAZARDS

Irritation Data: NA
Toxicity Data: NA

Section IV: PHYSICAL DATA

Appearance: Colorless liquid.

Section V: FIRE AND EXPLOSION HAZARD

Extinguishing Media: Carbon Dioxide, Dry Chemical Powder or Water Spray.
Special Firefighting Procedure: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Section VI: HEALTH HAZARD DATA

Acute Effects: Harmful if swallowed.
First Aid: In case of contact, flush eyes or skin with copious amount of water.
If swallowed, wash out mouth with water, provided person is conscious.
Call a physician.

Section VII: REACTIVITY DATA

Incompatibles: Anionic agents and nitrates.
Hazardous Combustion or Decomposition Product: Carbon Monoxide, Carbon Dioxide, Hydrogen Chloride Gas.

Section VIII: SPILL OR LEAK PROCEDURES

Steps To Be Taken if Material is Released or Spilled: Wipe spilled material with a dry cloth or absorb in sand. Place in a container for later disposal. Wash spill site after materials pick up is complete.
Waste Disposal Method: The substance can be disposed of as regular waste.

Section IX: PROTECTIVE EQUIPMENT AND CONTROL MEASURES

Wear chemical-resistant gloves, safety goggles and other protective clothing. Do not get in eyes, on skin or on clothing. Avoid prolonged or repeated exposure. Wash thoroughly after handling.

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Irritection® Assay System

Instruction Manual

Chapter 10
Irritection Assay System Y2K Update

InVitro International
17751 Sky Park East, Suite G
Irvine, California, 92614

IRRITECTION ASSAY SYSTEM Y2K UPDATE

I. The High Sensitivity kits are no longer available because we found that the regular Ocular kits can provide as good resolution as the High Sensitivity Ocular kits and at the same time provide an overall picture (over a scale of 0 – 51). The regular Dermal assay correlates to human data, while the High Sensitivity Dermal assay correlated to rabbit data. Therefore, we now encourage our customer to use the regular ocular as well as Dermal Irritection Assay System.

II. The Irritection software is compatible with the following plate readers: Dynex MRX, Dynatech MR600 / MR700, Dynatech MR5000 / MR7000, Molecular Devices Emax / Vmax / UVmax / THERMOmax, Cambridge 7520, Tecan and Thermal Lab Systems plate readers.

III. Starting in January 2000, the volume of Blanking Buffer in both the Ocular and Dermal kits has changed from 40mL to 30mL. Please note that the amount of activator added has changed from 800 µl to 600 µl and 2400 µl to 1800 µl respectively for Ocular cosmetic and surfactant kits (Refer to chapter 3, page 6 and page 30); For Dermal kits the amount of activator added has changed from 920 µl to 690 µl (Please refer to chapter 3, page 18 and 42).

IV. A few groups of chemicals are known to be difficult or even incompatible with the protein reagents that are utilized in IAS Ocular and Dermal Assays. An such example is the group of acidic formulations (pH < 2.0); another the alkaline formulations (pH >0.0). These have a tendency to cause false negative results. In addition, intensely colored materials get high OD readings for blanks and samples. For the successful management of these potential problems, please refer to Chapter 6.

V. A most frequent cause for a failed assay is not stirring the reagent wells thoroughly, especially the wells Cal2, Cal3, and QC2. Please use the wooden stirring sticks provided to scrape the reagent wells to ensure that all of the precipitate is removed from the bottom of each well and take care not to splash.