

## **IRRITECTION<sup>®</sup> ANALYTICS SOFTWARE INSTRUCTIONS**

Welcome to the Irritection Analytics, a program designed to make your data analysis processing easier and more productive. As long as your plate reader has 405 nm and 450 nm filters, you will be able to collect the optical density (OD) raw data and then use our software to perform data analysis and create a standard Irritection Assay report.

Prior to using the Irritection Analytics, make sure you have read the Irritection<sup>®</sup> Protocol(s) first.

### **Setting Up**

#### **A. Perform the Irritection<sup>®</sup> Assay**

1. On the first day, follow the Irritection<sup>®</sup> protocol to complete the assay set-up.
2. On the next day, follow the plate reader manufacturer instructions to set up your plate reader.  
Select a proper filter before reading the assay results.  
For Ocular Irritection<sup>®</sup> Assay: use 405 nm wavelength  
For Dermal Irritection<sup>®</sup> Assay: use 450 nm wavelength
3. Collect TWO sets of the raw 96-well plate data from your Plate Reader.

#### **SET ONE: Before Inhibiton Check**

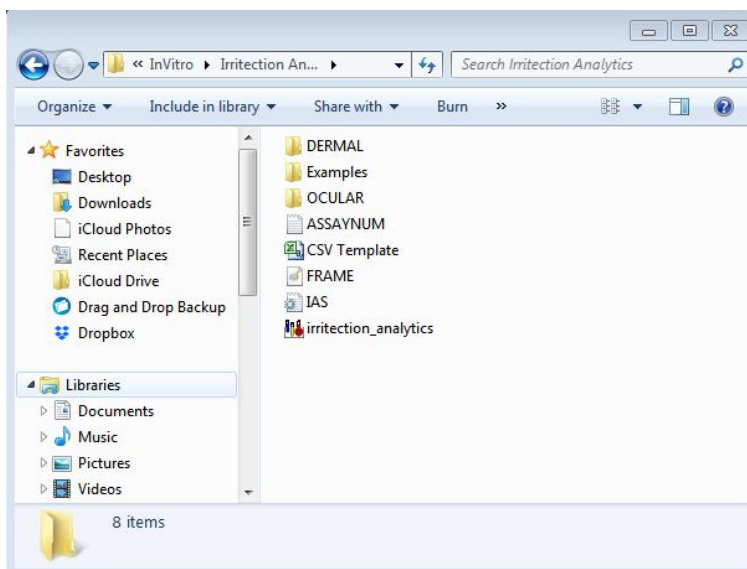
- a. After the 24-hour incubation period, follow the Irritection<sup>®</sup> Protocol to complete transferring the solutions from 24-well plate(s) to a 96-well reading plate.
- b. Read the 96-well reading plate on your plate reader and save the raw 96-well plate data.

#### **SET TWO: After Inhibiton Check**

- a. After finishing the first reading, add 25µl of Inhibition Check reagent to the following wells.  
For a 4-sample kit, add Inhibition Check to well C2, C3, C4, C5, C6, G2, G3, G4, G5, G6, C7, C8, C9, C10, C11, G7, G8, G9, G10 and G11.  
For a 3-sample kit, add Inhibition Check to well C2, C3, C4, C5, C6, G2, G3, G4, G5, G6, C7, C8, C9, C10 and C11.  
For a 2-sample kit, add Inhibition Check to well C2, C3, C4, C5, C6, G2, G3, G4, G5 and G6.  
For a 1-sample kit, add Inhibition Check to well C2, C3, C4, C5, and C6.
- b. Read the 96-well reading plate on your plate reader and save the raw 96-well plate data.

**B. Create a CSV file**

1. Open the “CSV Template” file in C:\Programs\InVitro\Irritection Analytics.




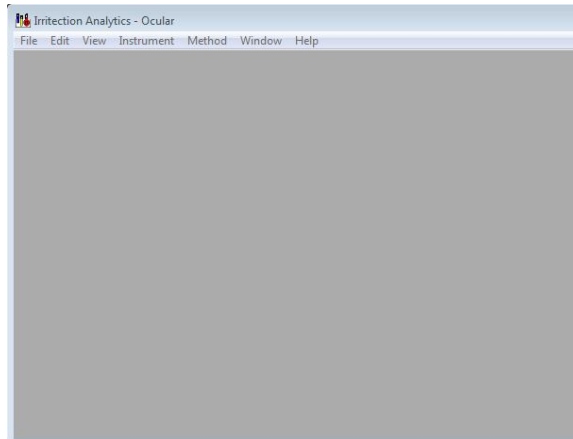
2. Copy and paste the *SET ONE* raw 96-well plate data in the area highlighted in red below.  
Copy and paste the *SET TWO* raw 96-well plate data in the area highlighted in blue below.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Copy and Paste the raw 96-Well Plate Data in the Area Below													
2														
3	Plate Data 1: Before Inhibition Check													
4	*	1	2	3	4	5	6	7	8	9	10	11	12	
5	A													
6	B													
7	C													
8	D													
9	E													
10	F													
11	G													
12	H													
13														
14	Plate Data 2: After Inhibition Check													
15	*	1	2	3	4	5	6	7	8	9	10	11	12	
16	A													
17	B													
18	C													
19	D													
20	E													
21	F													
22	G													
23	H													
24														

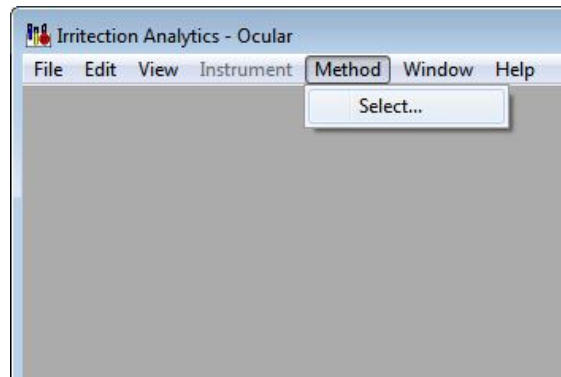
3. Once you have finished transferring data, rename and save the file. Make sure the file saves as a CSV file. You can find examples under C:\Programs\InVitro\Irritection Analytics\Examples.

## Launching the Irritection Analytics Software

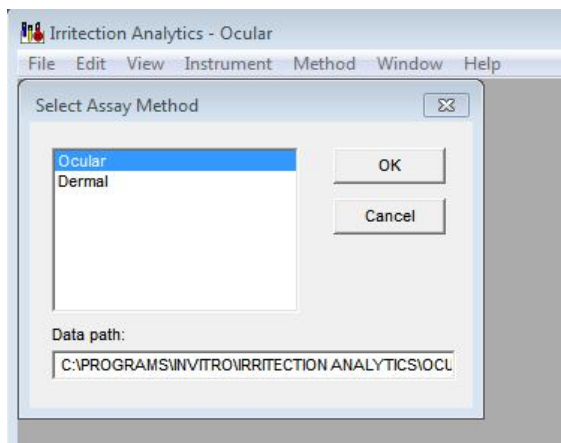
1. Double-click on the Irritection Analytics  icon to launch the program.
  - The command window appears when you launch the software. This is the main window for the Irritection Analytics Software.



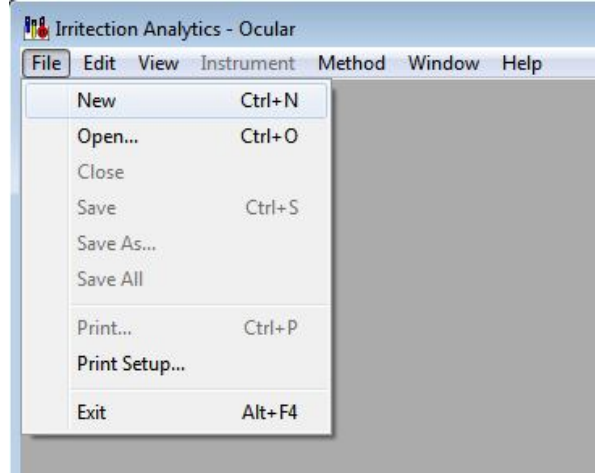
2. Select the Assay Method
  - a. From the *Method* menu, choose *Select*.



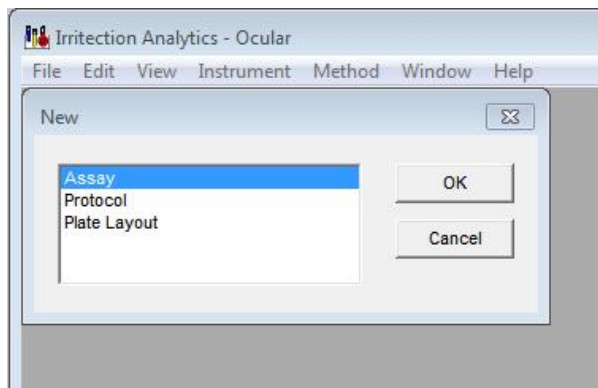
- b. Select the desired assay method (Ocular or Dermal) and click the *OK* button.



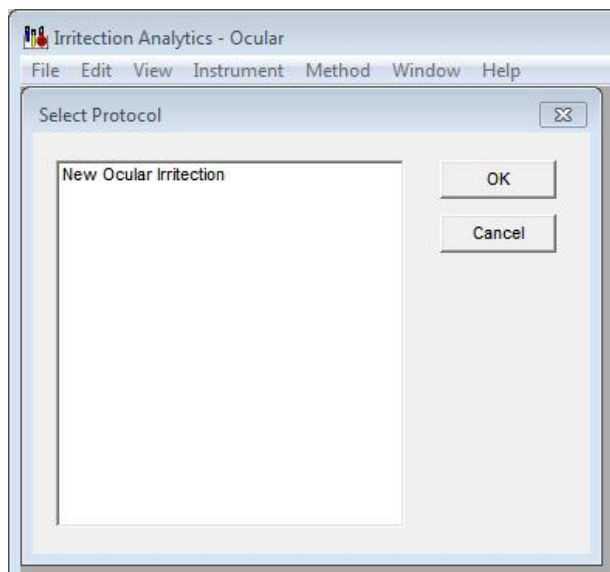
3. Reading a New Assay
  - a. From the *File* menu, choose *New*.



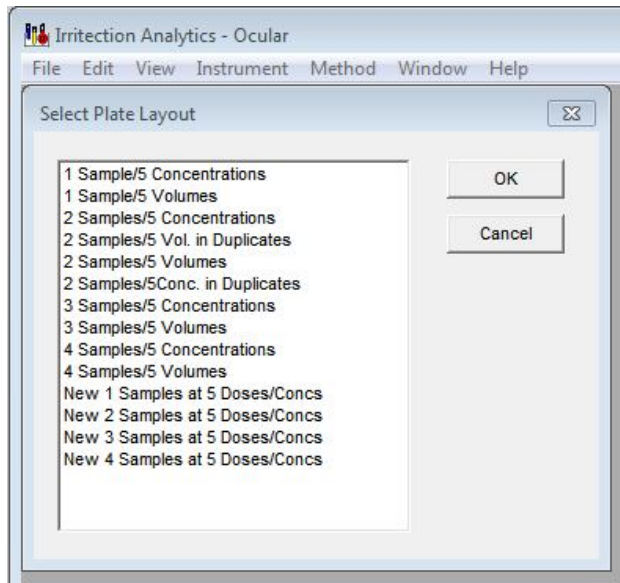
- b. Select *Assay*, and click *OK* button.



- c. Select the desired protocol, and click the *OK* button.

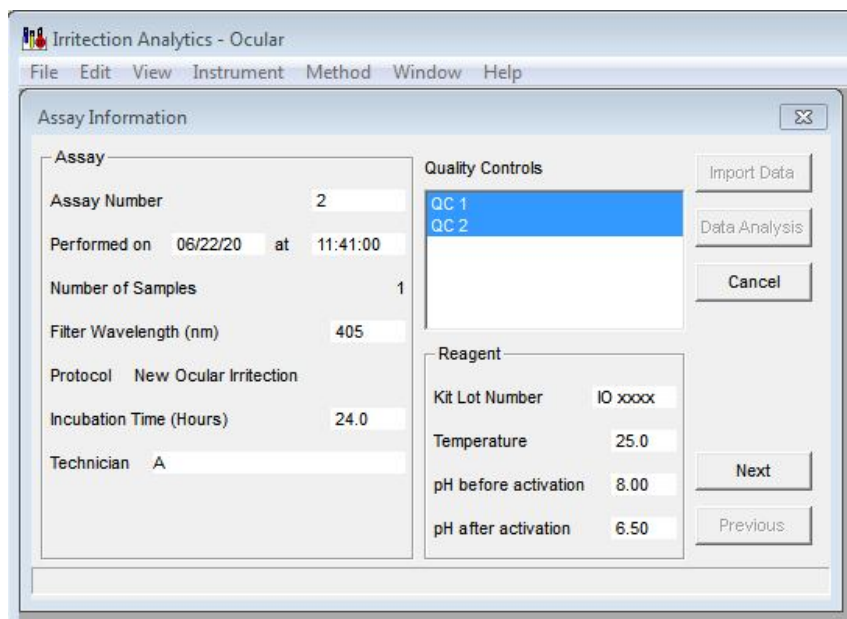


d. Select the desired plate layout, and click the *OK* button.



e. A series of screens will be displayed. You will need to enter the appropriate assay, and sample information.

- Enter the Incubation Time, Technician, Kit lot Number, Temperature, pH before activation and pH after activation. Once you have finished entering the assay information, Click the *Next* button.



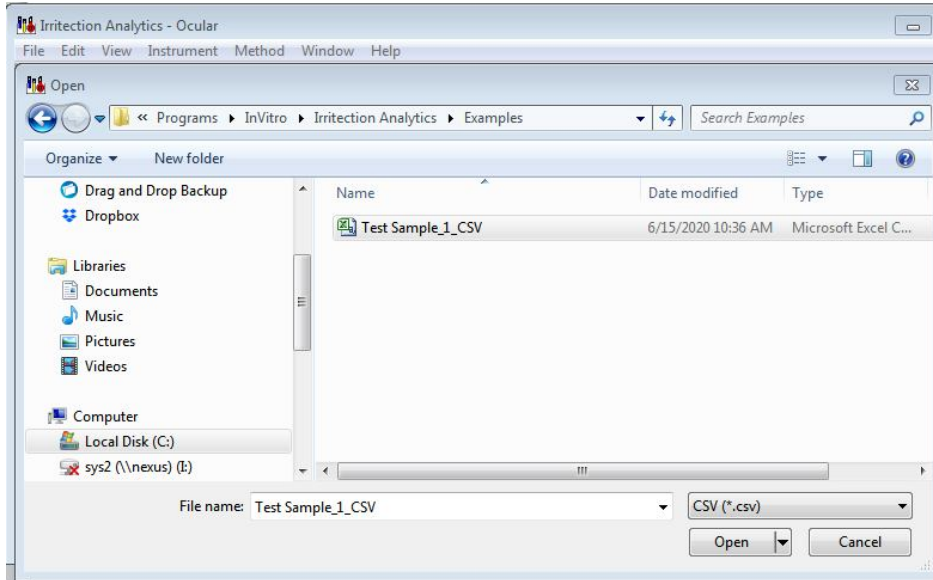
- Enter the Description, Product Type, pH and Sample number. Once you have finished entering the sample information, Click the *Next* button.

**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.**

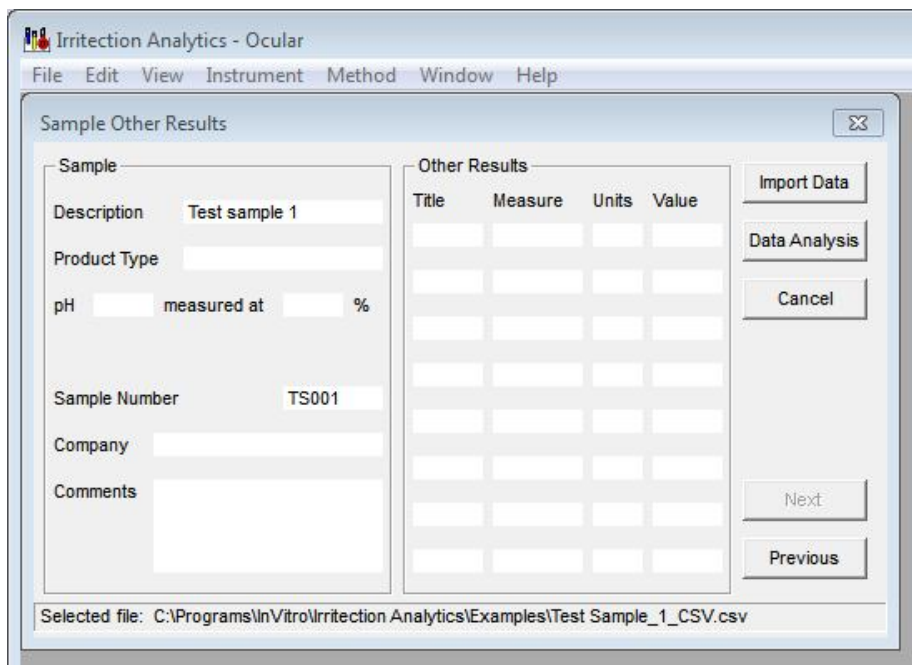
- No need to enter any information in the following dialog window.

- Once you have finished entering the information for each sample, Click the *Import Data* button.

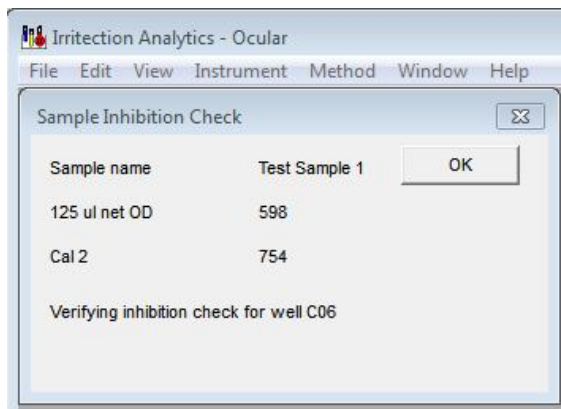
- The following dialog window will appear. Select the CSV file you've created for the assay results, and click the *OPEN* button.



- The following dialog window will appear with the selected file path at the bottom of the window. Click *Data Analysis* button.



- During the data analysis, a dialog box will be displayed if an “Inhibition Check” verifying step is required. Click the *OK* button to continue the process.



- After the data analysis has been completed, a dialog box will be displayed indicating if the assay was qualified or unqualified. Press the *OK* button to continue.

**ASSAY REPORT - ORIGINAL**

Sample Description :	Test Sample 1	Date :	06/22/20
Sample Number :	TS001	Time :	11:41:00
Product Type :		Technician Name :	A
Assay Method :	Ocular	Kit Lot Number :	IO xxxx
Protocol :	New Ocular Irritation	Reagent temperature :	25.0
Incubation Time :	24.0 hours	Reagent pH Before Activation :	8.00
Plate Layout :	1 Sample/5 Volumes	Reagent pH After Activation :	6.50
Instrument Type :	Raw Data	Sample pH at 10% :	8.00
Wavelength :	405nm	Assay Number :	2
Comment :		Assay Qualification :	Qualified

**Sample Results:**

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
25 ul	188	10	178	11.8	Minimal/Mild	Qualified
50 ul	196	20	176	11.7	Minimal/Mild	Qualified
75 ul	389	24	365	18.0	Mild	Qualified
100 ul	623	24	599	25.2	Mild	Qualified
125 ul	624	26	598	25.2	Mild	Qualified

**Calibrator Values:**

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
Cal 0	106	0.0	62 - 262	Range qualified

#### 4. Viewing/Printing Assay Results

- 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.
- To print the complete processed report, go to the *File* menu, and click the *Print* button. (The defaults have already been selected for you.)
- To print additional sections of the report, select the section, and click on the *Print* button.