User Guide
To the Luminescence Macro
(Designed for Five Samples per Plate)

The purpose of this user guide is to provide easy instructions on how to use the luminescence macro for data analysis in the TZM-bl neutralizing antibody assay.

I. Introduction

Overview

The luminescence macro is a series of commands and functions embedded in an Excel workbook that can automate a repetitive task for rapid data reduction.

Once the macro is properly installed, it can be initiated whenever the task needs to be performed.

The luminescence macro can:

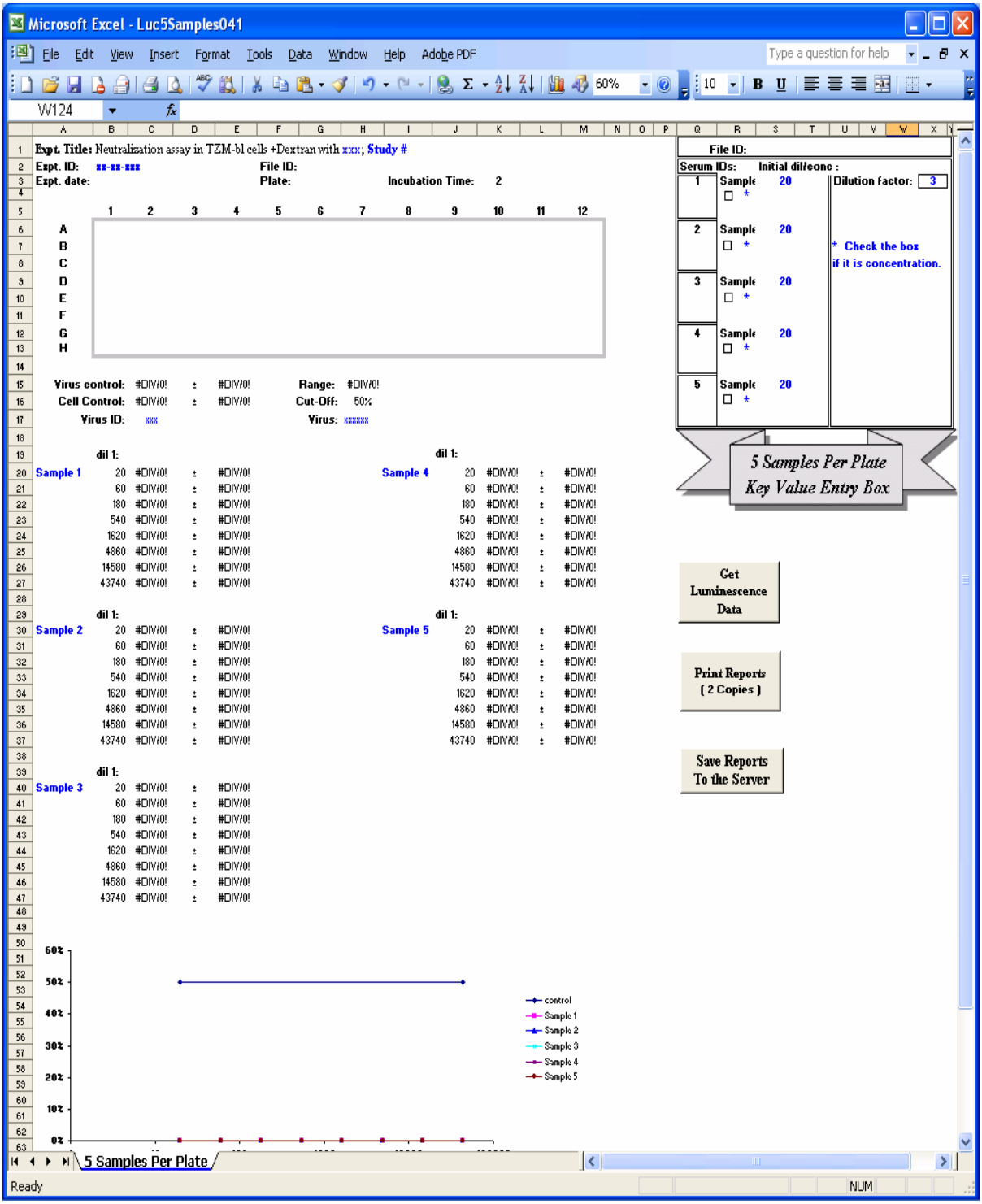
- ***Calculate the percent neutralization for each serum dilution.***
- ***Calculate neutralizing antibody titer.***
- ***Create a chart that plots neutralization curves for each sample***

The full name of this macro program file is: **Luc5samples04.xlt.**

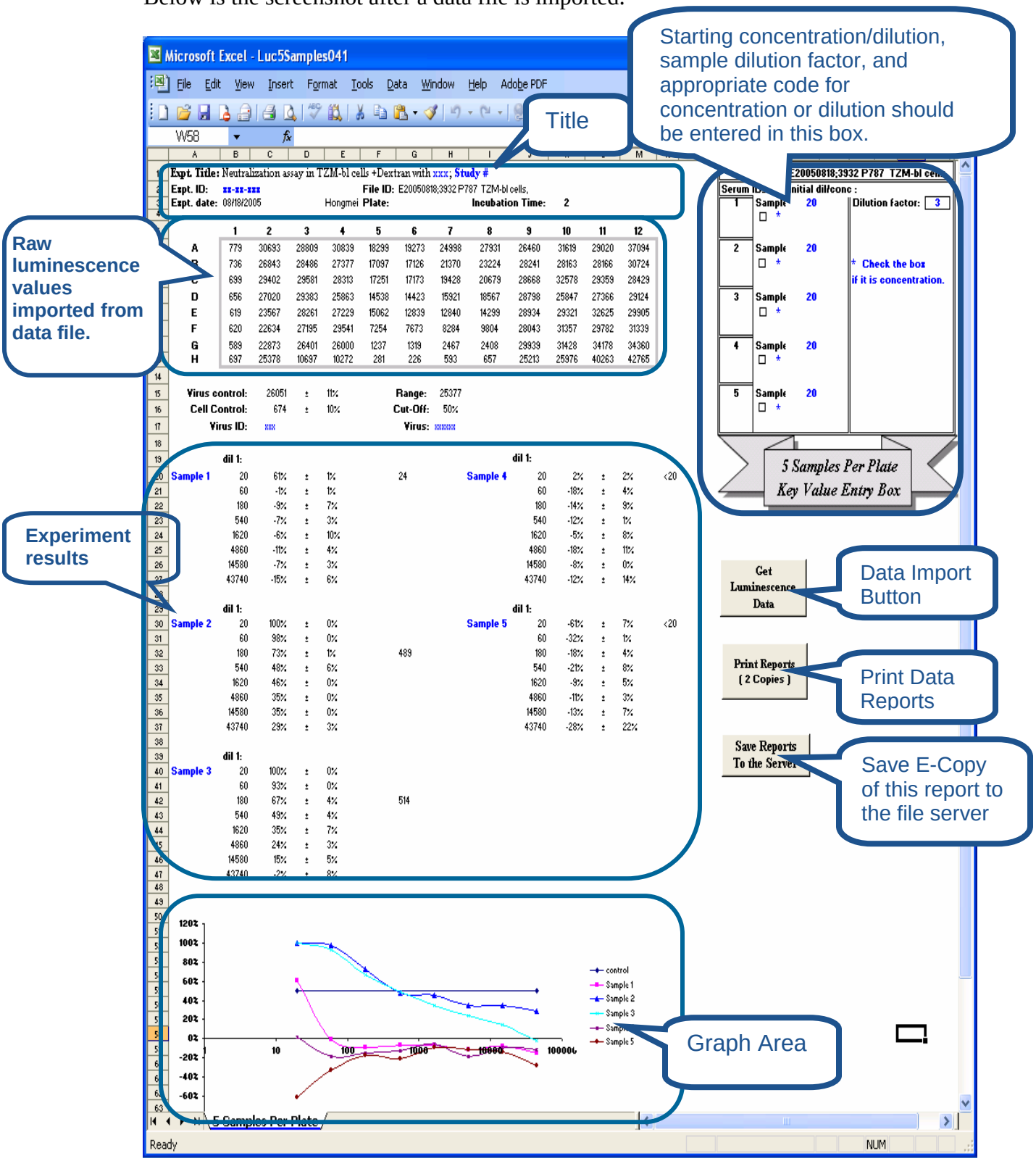
- **Luc** is short for ***Luciferase assay***
- **5** refers to ***5 samples per plate***
- **04** is ***the macro version number***

Features

Below is the screenshot of a blank macro file when you open it.

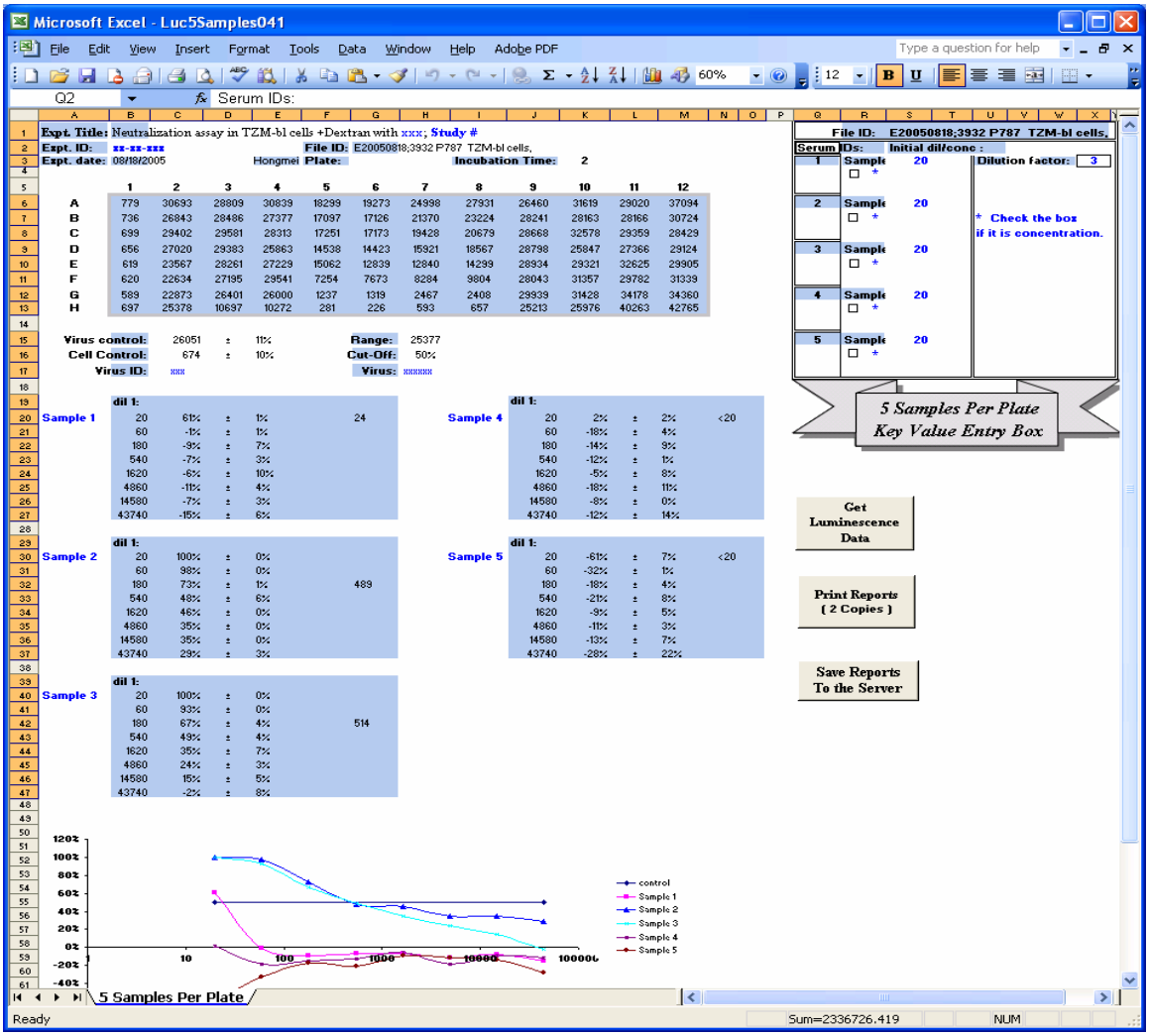


Below is the screenshot after a data file is imported.



Security Feature

In order to protect the worksheet from accidental changes, information in the shades areas (see picture below) are locked with password protection.



Any attempt to enter information in a restricted cell (shown as shade section above) will give you a warning dialog box (see below).

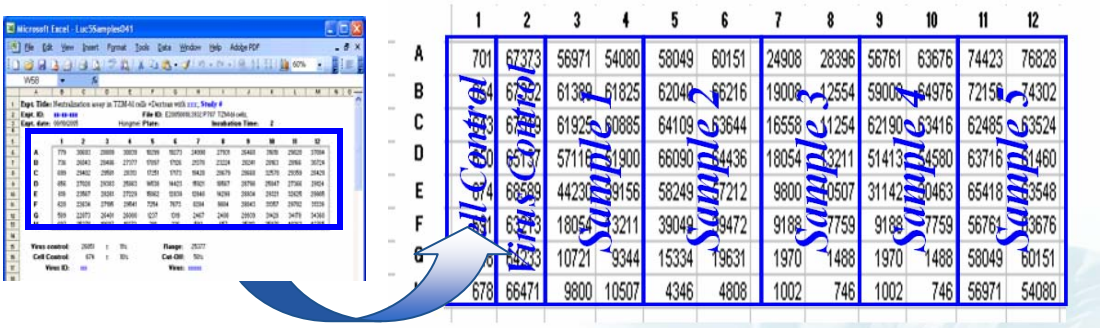
Template of 5 Samples per Plate

The templates for 5 samples per plate neutralization assays

	1	2	3	4	5	6	7	8	9	10	11	12
A	CC	VC	Dil 8	Dil 8	Dil 8	Dil 8	Dil 8	Dil 8	Dil 8	Dil 8	Dil 8	Dil 8
B	CC	VC	Dil 7	Dil 7	Dil 7	Dil 7	Dil 7	Dil 7	Dil 7	Dil 7	Dil 7	Dil 7
C	CC	VC	Dil 6	Dil 6	Dil 6	Dil 6	Dil 6	Dil 6	Dil 6	Dil 6	Dil 6	Dil 6
D	CC	VC	Dil 5	Dil 5	Dil 5	Dil 5	Dil 5	Dil 5	Dil 5	Dil 5	Dil 5	Dil 5
E	CC	VC	Dil 4	Dil 4	Dil 4	Dil 4	Dil 4	Dil 4	Dil 4	Dil 4	Dil 4	Dil 4
F	CC	VC	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3
G	CC	VC	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2
H	CC	VC	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1
			Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	

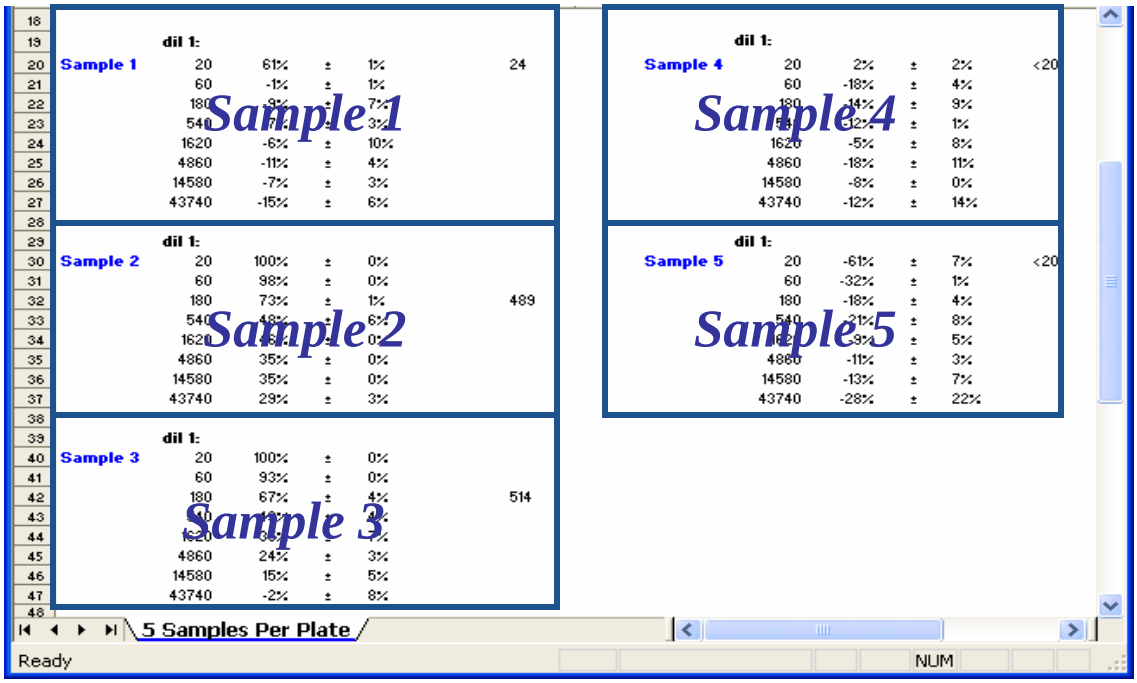
CC: cell control wells (cells only)
VC: virus control wells (virus and cells but no serum sample are added here)
Dil: Dilution

Plate set-up in the protocol

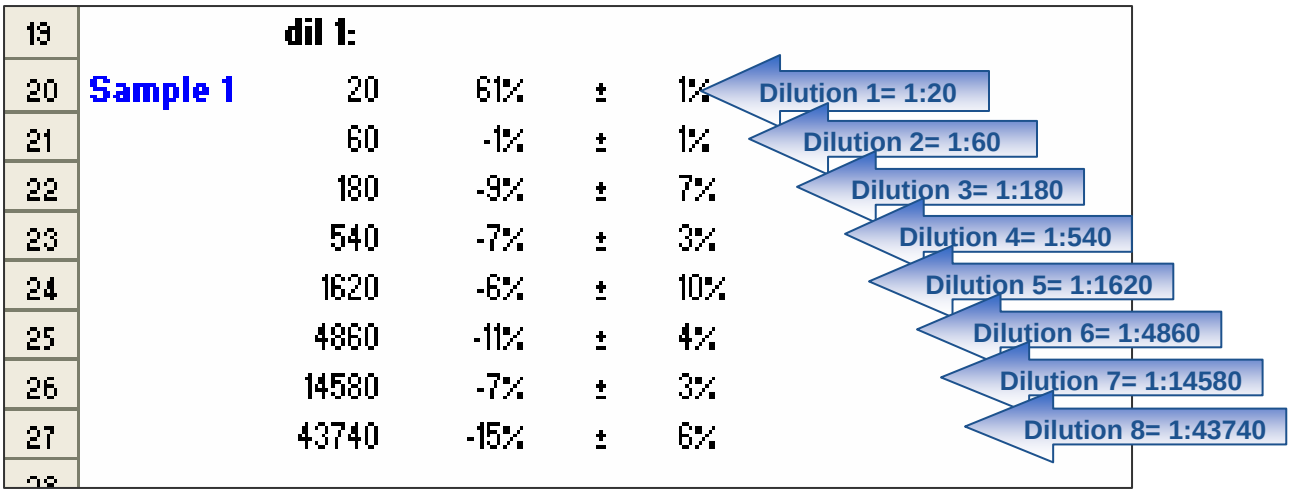


(In the program file)

Below are the positions of the 5 samples in the experiment results area.

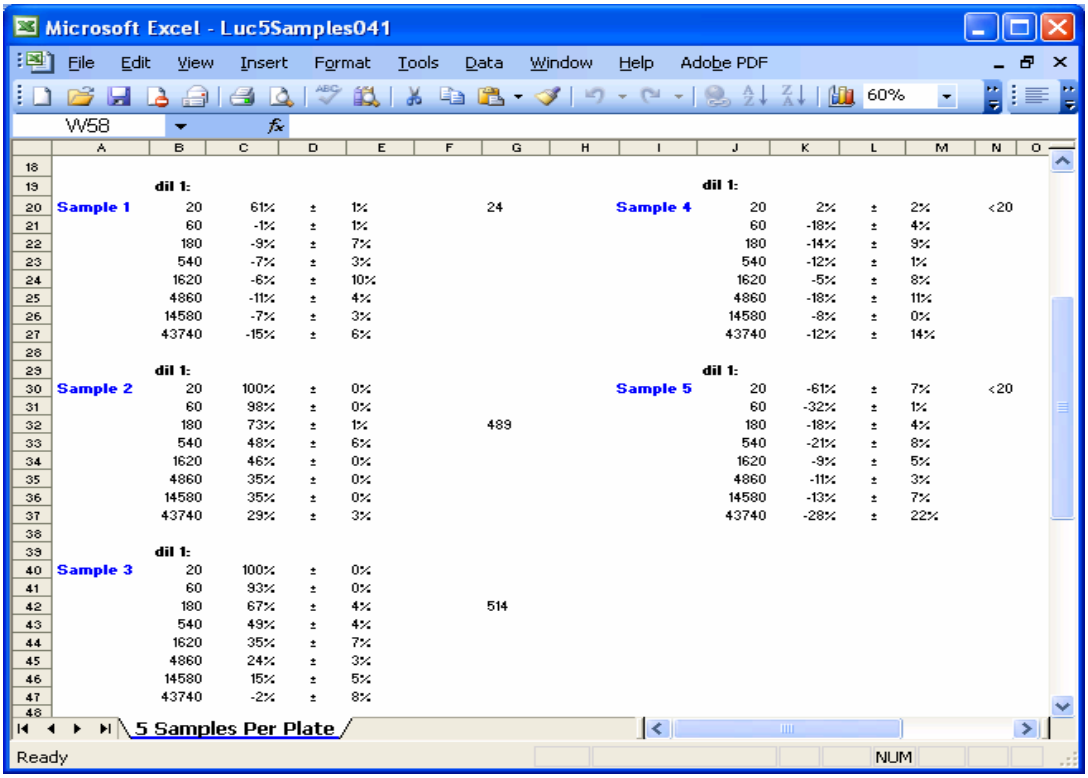


Let's take sample 1 as an example. From the top to bottom are dilutions 1 - 8.



Note: dil stands for dilution.

Column Information

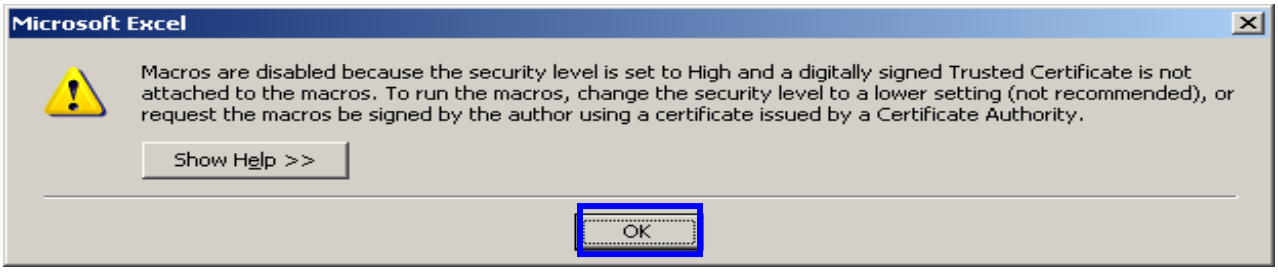


Column A and I: ***Sample name, bleed date, etc***
Column B and J: ***Dilution or concentration of each test sample***
Column C and K: ***Neutralization Efficiency (%) for each sample***
Column E and M: ***Standard Deviation of Neutralization Efficiency***
Column F, G and N, O: ***Showing titer value (dilution or concentration)***

II. Configuration

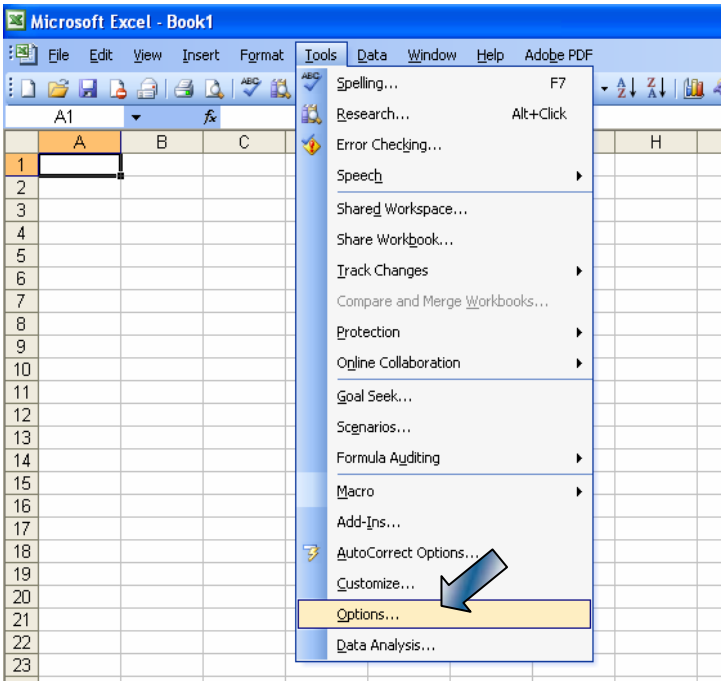
Macro Security Setting Change

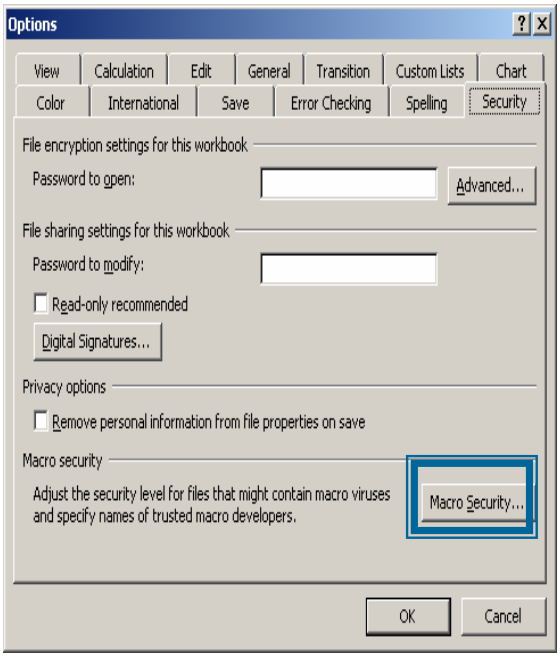
The first time you start the luminescence macro program, you may encounter the following dialog box, which prompts that you need to change Excel Macro security settings.

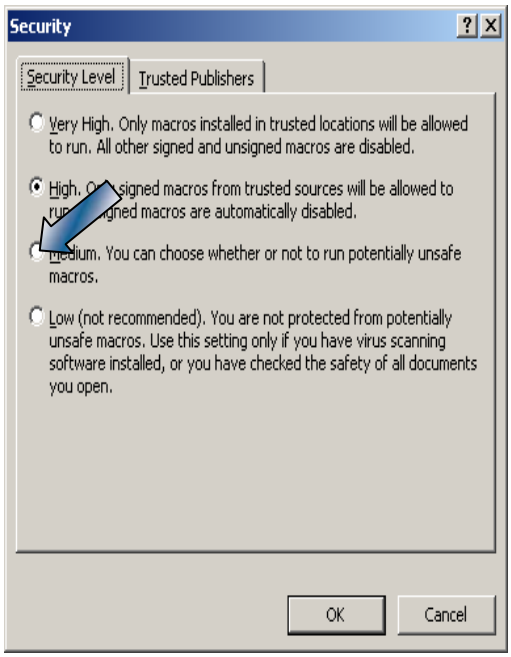


To change the macro security level, please follow these steps.

1. Click **OK** button in the above dialog box.
2. On the **Tools** menu, click **Options**.
3. Click the **Security** tab.
4. Under **Macro Security**, click **Macro Security**.
5. Click the **Security Level** tab, and select **Medium**.
6. Click **Ok** to close the dialog boxes.







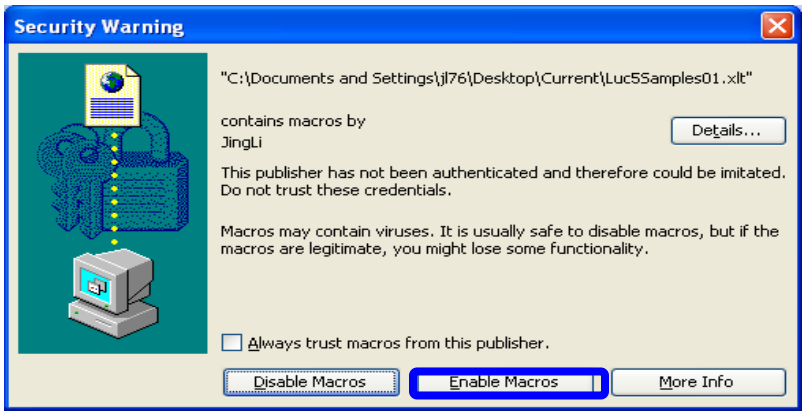
Close and then restart the Excel program. The disabled macros message should not appear.

After this configuration is completed, it will not be necessary to change the macro security settings each time you use the macro.

III. Step by Step

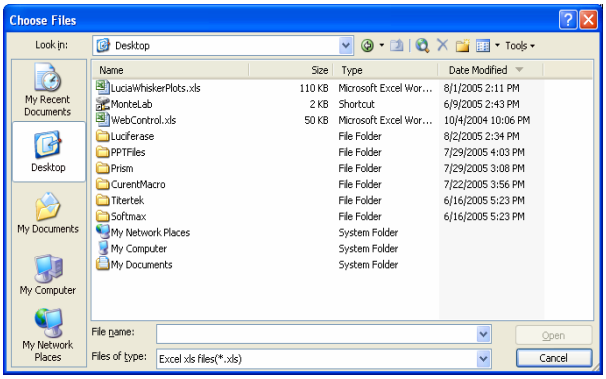
1. Open the macro file entitled **“Luc5Samples04.xlt”**.

2. Click **“Enable Macros”.**



After you open the macro file, the data area is blank waiting for data input.

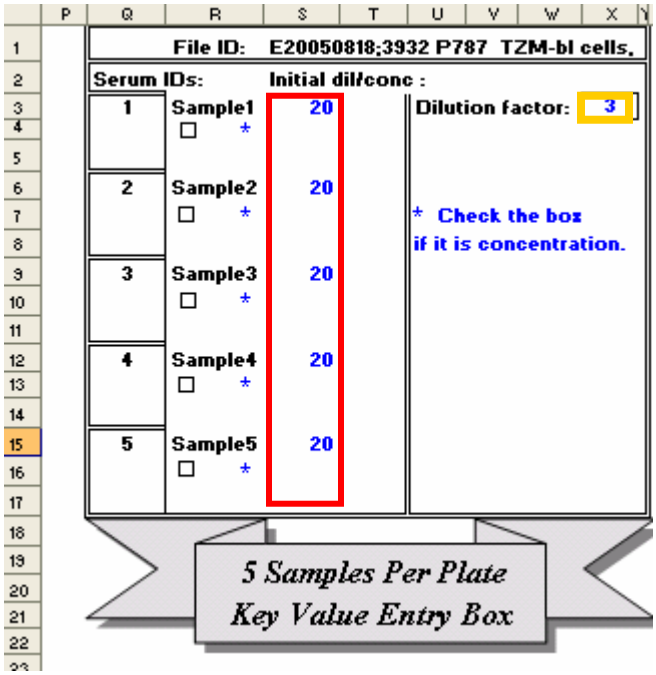
3. Click the **“Get Luminescence Data”** button to open TITER data file.



After click “Get Luminescence Data”, **Choose Files** Dialog box appears. Locate your luminescence raw data file.

4. Enter appropriate sample **starting dilutions** (or **concentrations**) and **dilution factor**.

Enter the starting dilution and dilution factor directly as indicated below.



Enter a value in **Column S** (indicated by red rectangle) to set the starting dilution or concentration value.

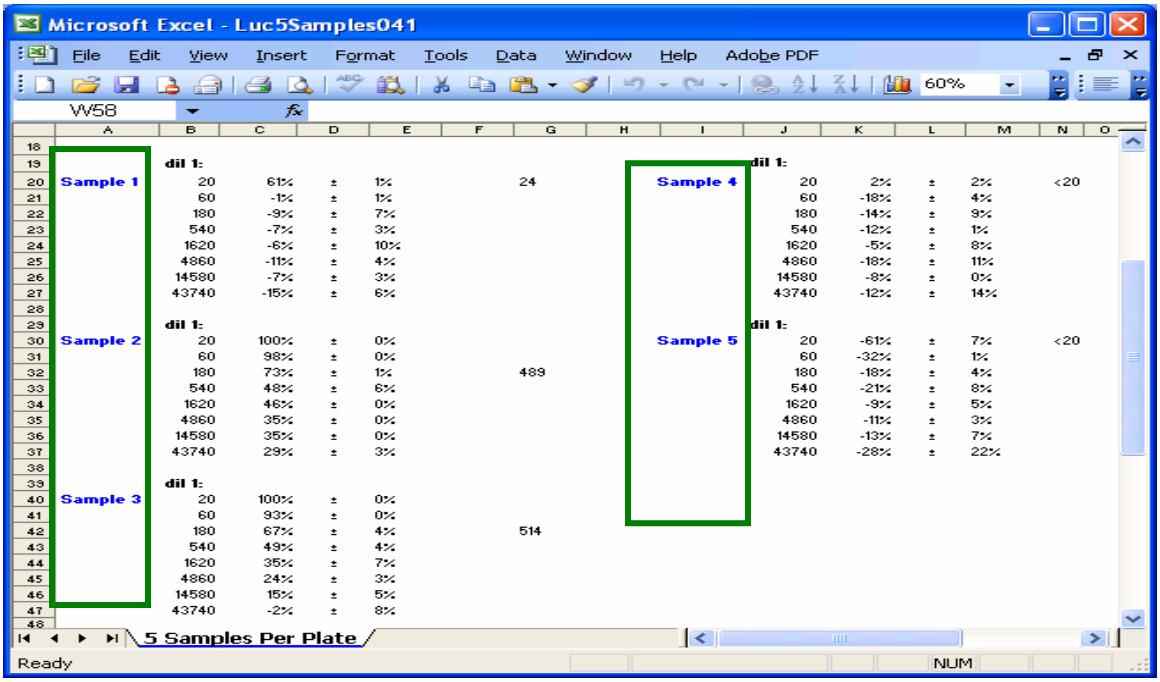
Enter a value in cell **"X3" (**indicated by yellow rectangle) to set the dilution factor.

5. Check the box next to the corresponding samples that have a known **concentration**.

The macro has separate functions that allow samples to be analyzed either as dilutions (e.g., serum and plasma) or concentrations (e.g., MAbs, µg/ml). No action needs to be taken if all 5 samples are to be analyzed as dilutions

6. Enter additional experiment Information in the Title section.
(e.g. Experiment title, Experiment ID, Study No, Incubation time.)

7. Enter **general sample information** in column A and column I indicated by green rectangles.
(e.g. Sample name, bleed number for each sample.)



8. Print out data and save an electronic copy of the report file on the server.

Click this button to get two copies of your data report. One for the Principal Investigator, the other one will be kept by the technician.

9. Click “Get Luminescence Data” button to open another data file or close the macro program without saving any change.

Help

Please feel free to contact **Jing Li** ((*jing.li@duke.edu*) if you have any question about this guide or encounter any problems. Any suggestions for improvement are also welcome.

Last updated: 9-18-06