User Guide to the Luminescence Macro

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

Download Source

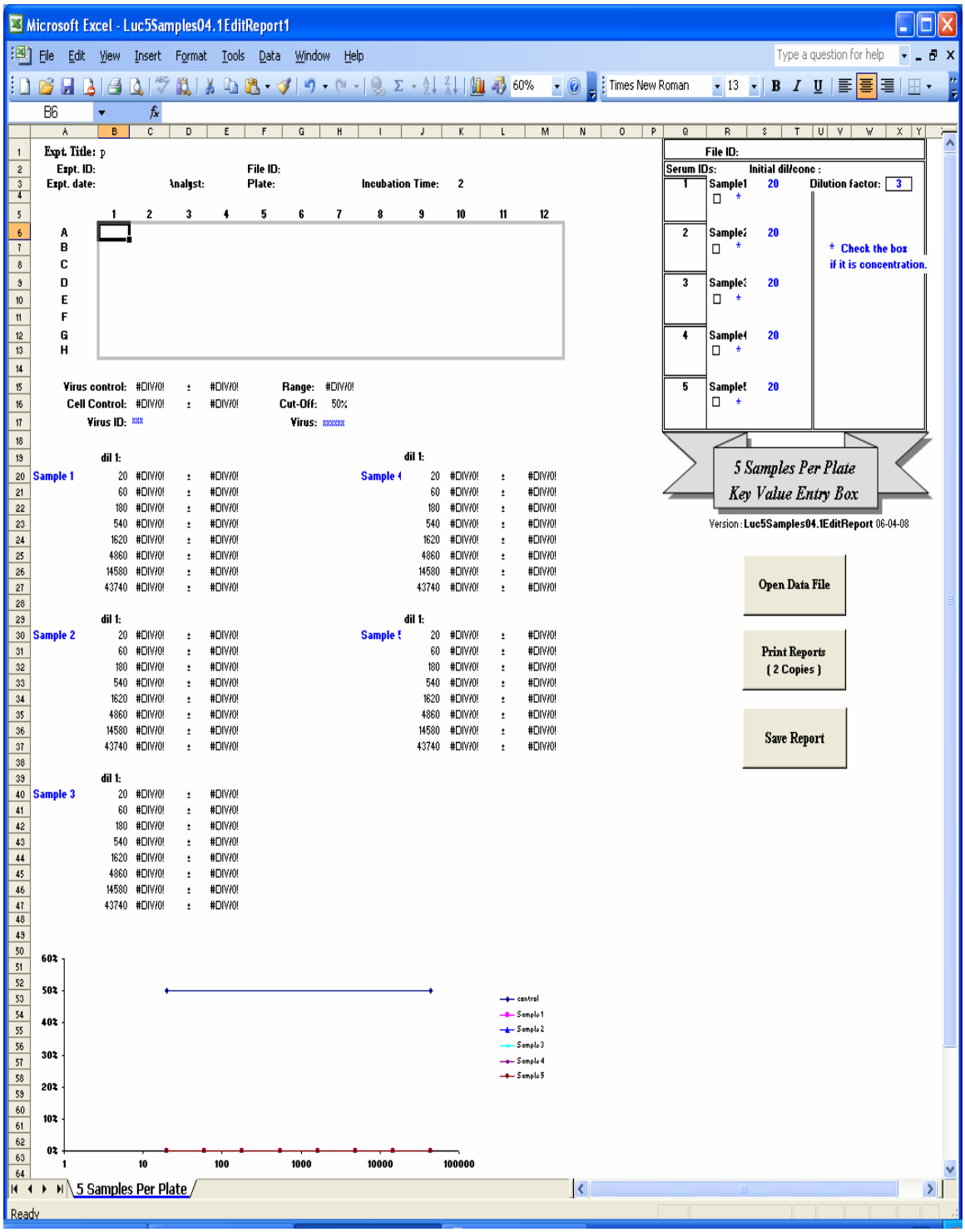
The luminescence macro can be downloaded from the following website:
http://www.hiv.lanl.gov/content/nab-reference-strains/html/home.htm

The full name of this macro program file is: **Luc5samples04xxx.xlt.**

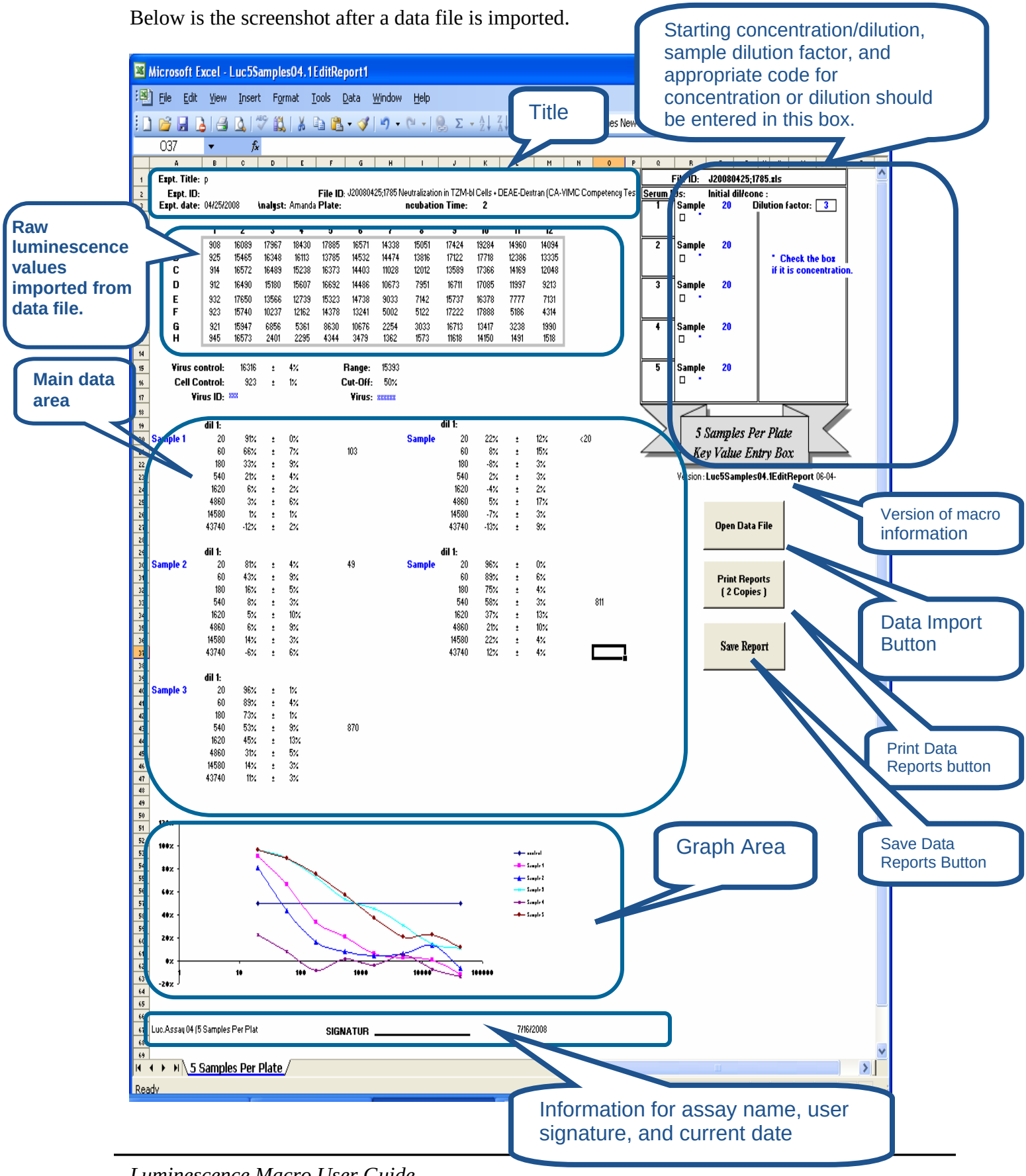
- **Luc** is short for ***Luciferase assay***
- **5** refers to ***5 samples per plate***
- **04xxx** is ***the macro version number (Current version is Luc5samples04.vEditReport***

Features

Below is the screenshot of a blank macro file after opening the macro.

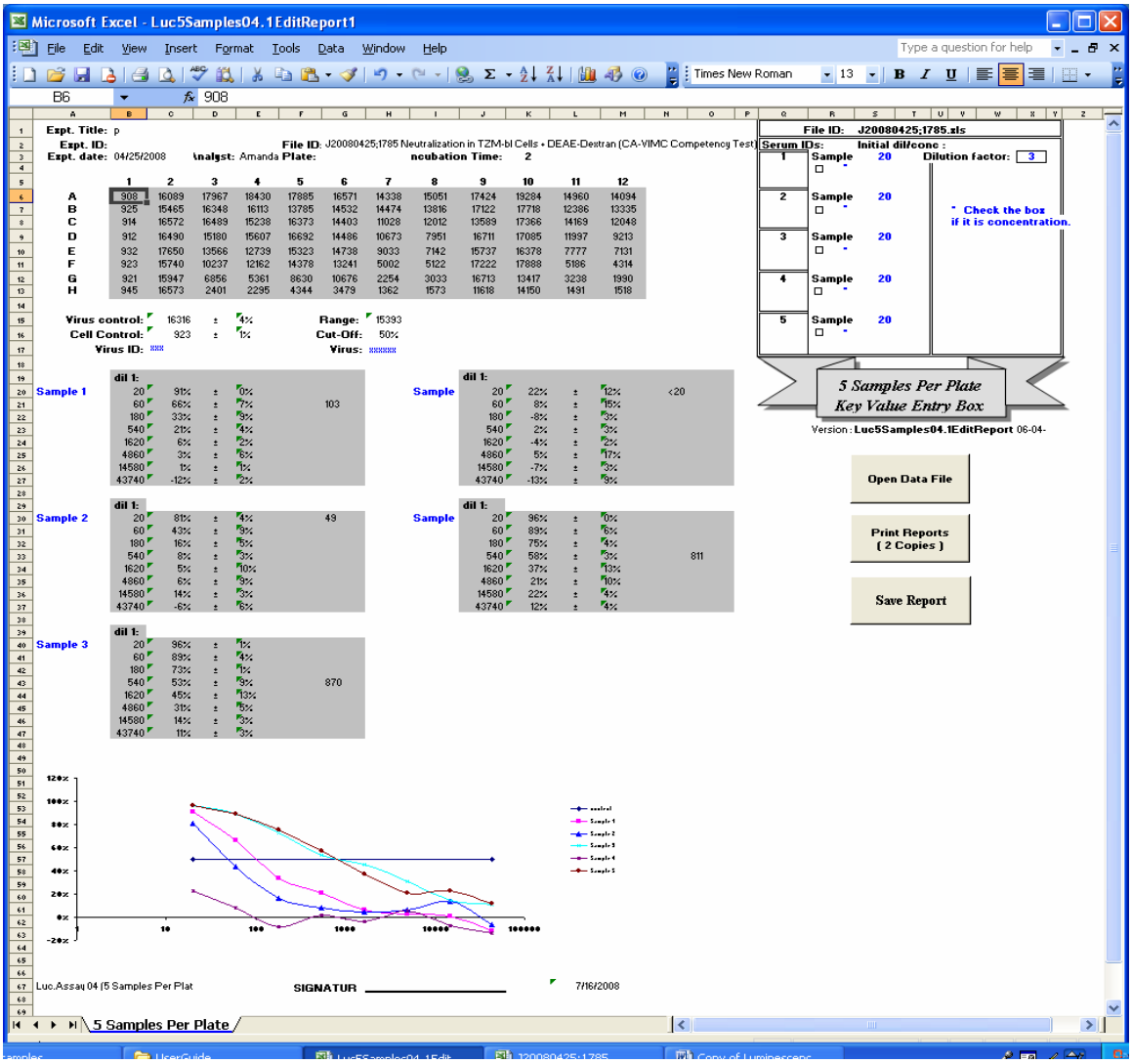


Below is the screenshot after a data file is imported.



Security Feature

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



A warning dialog box (see below) will appear with each attempt to enter information in a restricted cell (shown as a shades section above).

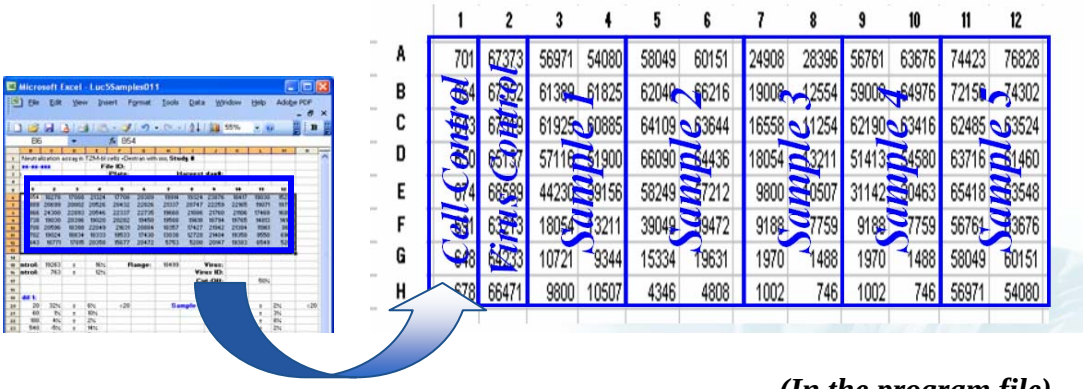
Template of 5 Samples per Plate

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	

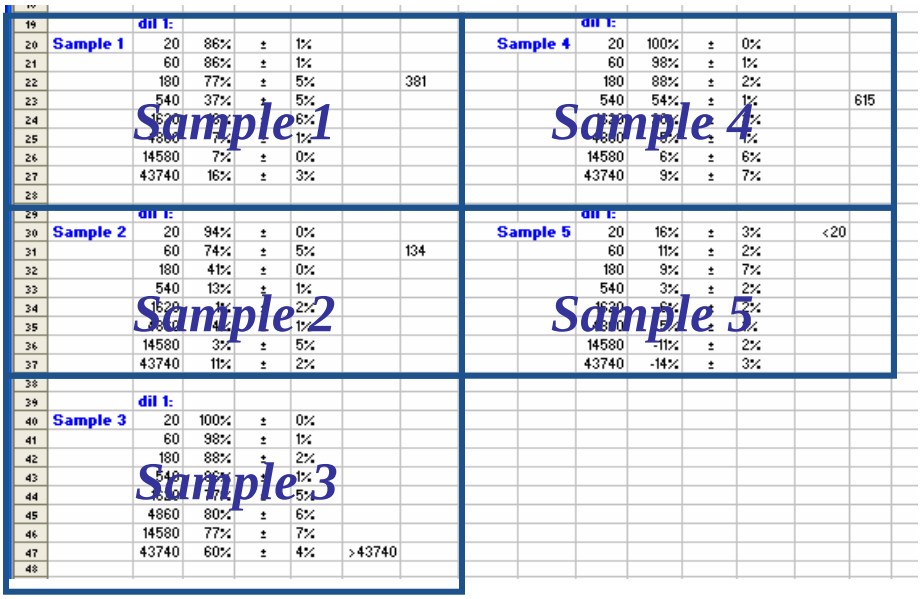
CC: cell control wells (cells only)
VC: virus control wells (virus and cells but no serum sample are added here)
Dil: Dilution

Plate Set-up

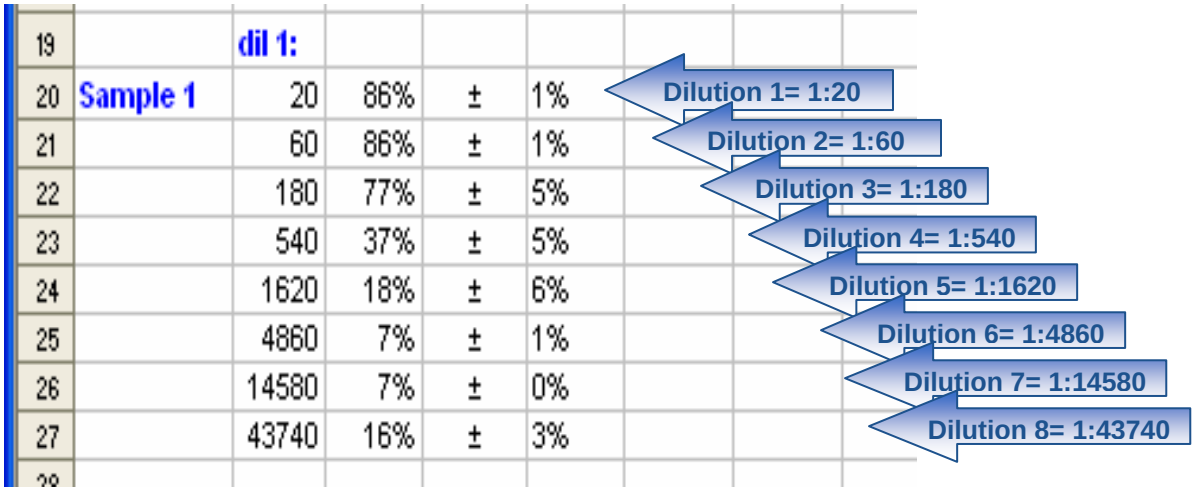


(In the program file)

Below are the positions of the 5 samples in the main data area.

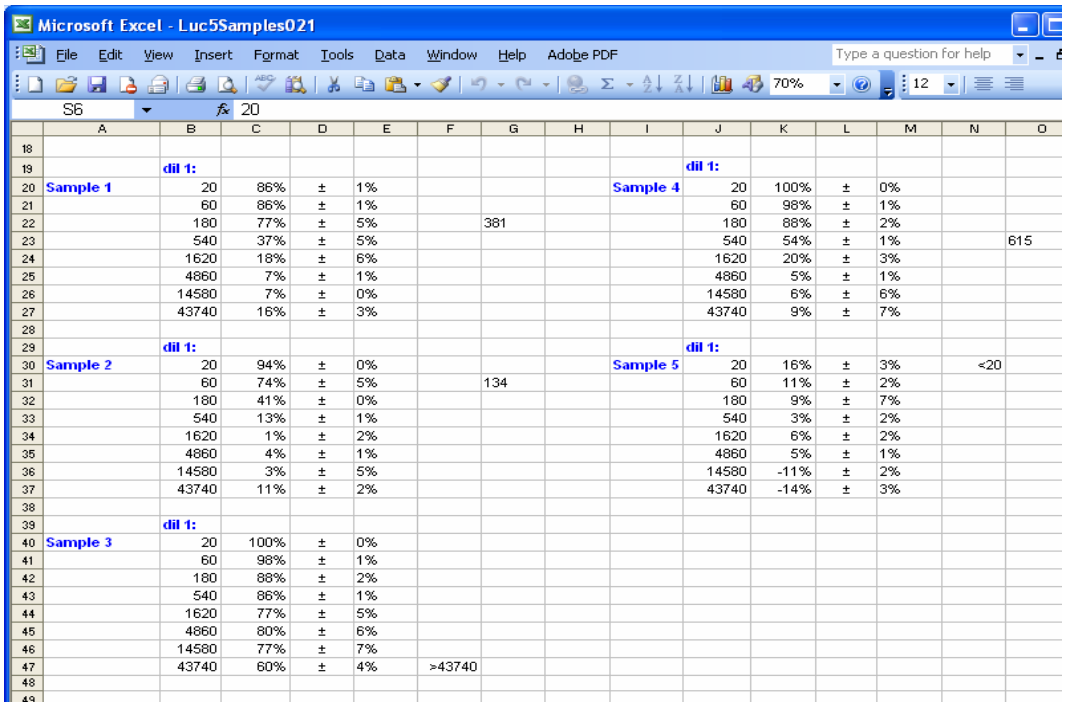


Let's take sample 1 as an example. From the top to bottom are dilutions 1 - 8.



Note: dil stands for dilution.

Column Information



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
18															
19		dil 1:								dil 1:					
20	Sample 1	20	86%	±	1%				Sample 4	20	100%	±	0%		
21		60	86%	±	1%					60	98%	±	1%		
22		180	77%	±	5%		381			180	88%	±	2%		
23		540	37%	±	5%					540	54%	±	1%		615
24		1620	18%	±	6%					1620	20%	±	3%		
25		4860	7%	±	1%					4860	5%	±	1%		
26		14580	7%	±	0%					14580	6%	±	6%		
27		43740	16%	±	3%					43740	9%	±	7%		
28															
29		dil 1:								dil 1:					
30	Sample 2	20	94%	±	0%				Sample 5	20	16%	±	3%	<20	
31		60	74%	±	5%		134			60	11%	±	2%		
32		180	41%	±	0%					180	9%	±	7%		
33		540	13%	±	1%					540	3%	±	2%		
34		1620	1%	±	2%					1620	6%	±	2%		
35		4860	4%	±	1%					4860	5%	±	1%		
36		14580	3%	±	5%					14580	-11%	±	2%		
37		43740	11%	±	2%					43740	-14%	±	3%		
38															
39		dil 1:													
40	Sample 3	20	100%	±	0%										
41		60	98%	±	1%										
42		180	88%	±	2%										
43		540	86%	±	1%										
44		1620	77%	±	5%										
45		4860	80%	±	6%										
46		14580	77%	±	7%										
47		43740	60%	±	4%	>43740									
48															

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

Data File

The new version of the macro can read the Luminometer Raw Data File and as well as modify any report file which was previously generated.

1 Luminometer Raw Data File

The luminometer data file may be saved with a name in the format of Xmmddyy$$;####.xls.

Raw data are generated from the luminometer using the Wallac 1420 software package from PerkinElmer Life Sciences). These raw files will be exported individually from the Wallac1420 Manager and renamed in the format of "Xmmddyy$$;####.xls" .

- X: a unique character assigned to the assay operator
- mm: current month
- dd: current day
- yy: current year
- $$: plate number(1-99)
- #### : Four digit unique number automatically assigned by the luminometer

For example: A07220512;3767 corresponds to the twelfth plate read by operator " A" on July 2 , 2005. The 3767 corresponds to the unique number assigned by the luminometer.

If the data file is saved in the format of "Xmmddyy$$;####.xls", some information (like Experiment Date, plate number, File ID) indicated by the red boxes below in the Title Part will be automatically filled in.

	A	B	C	D	E	F	G	H	I	J	K
1	**Expt. Title:**	Neutralization assay in TZM-bl cells +Dextran with xxx; **Study #**									
2	**Expt. ID:**	xx-xx-xxx				**File ID:**	X07270515;3777 DP6-002 TZM-bl cells				
3	**Expt. date:**	7/27/05				**Plate:**	15		**Incubation Time:**		
4											

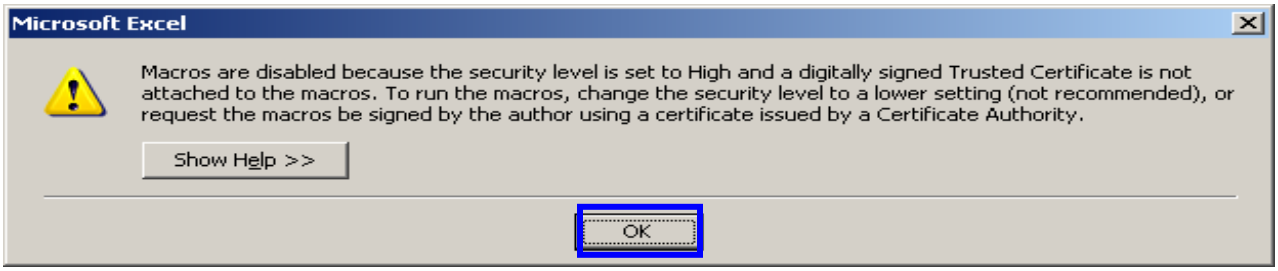
2 Report File

An operator may want to modify the report at a later time due to typographic errors. This version of the macro allows operators to modify the input fields in data report file. Using Open Data File button, select the report name to be opened and then click it. The macro will input the report data to the macro.
Note: If the name of the report does not include "report", the macro can not open the report automatically.
Please note that the raw and analyzed data section is still locked and cannot be modified!

II. Configuration

Macro Security Setting Change

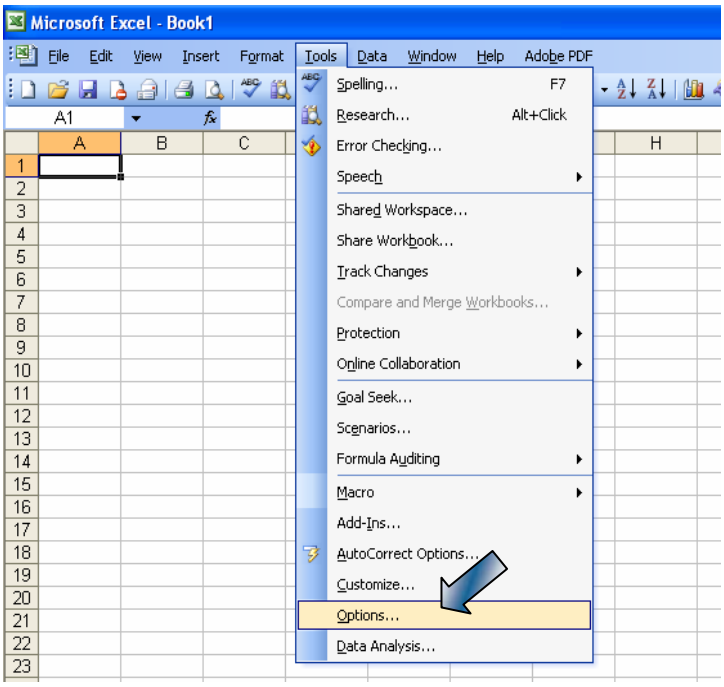
The first time the luminescence macro program is opened, the following dialog box may appear, prompting the operator to change the Excel Macro security settings.

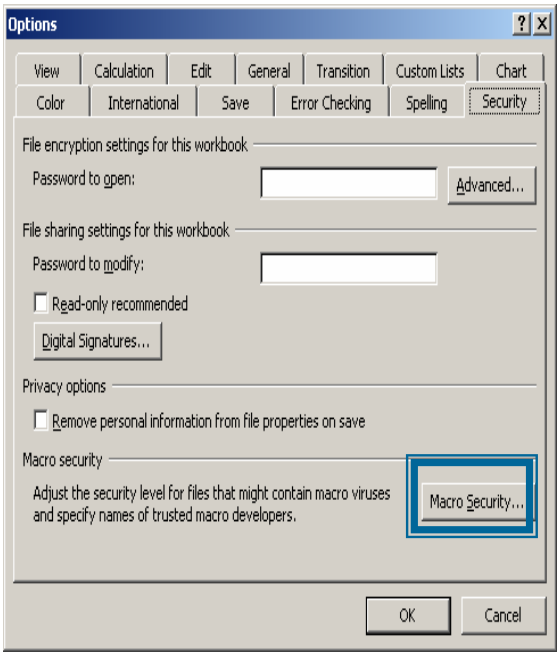


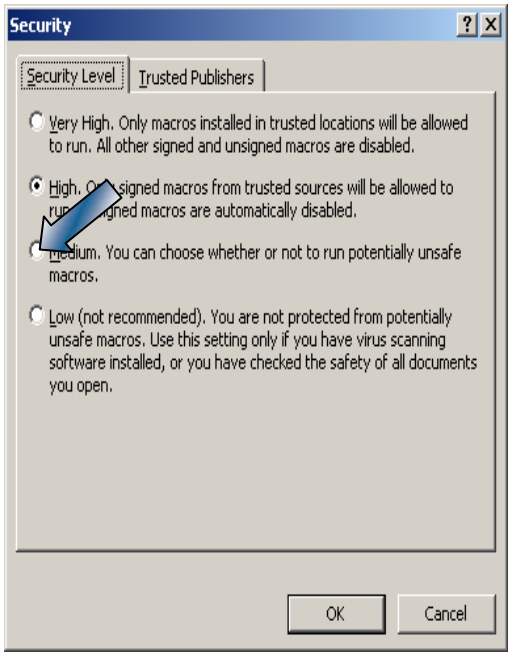
Windows XP Users:

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.

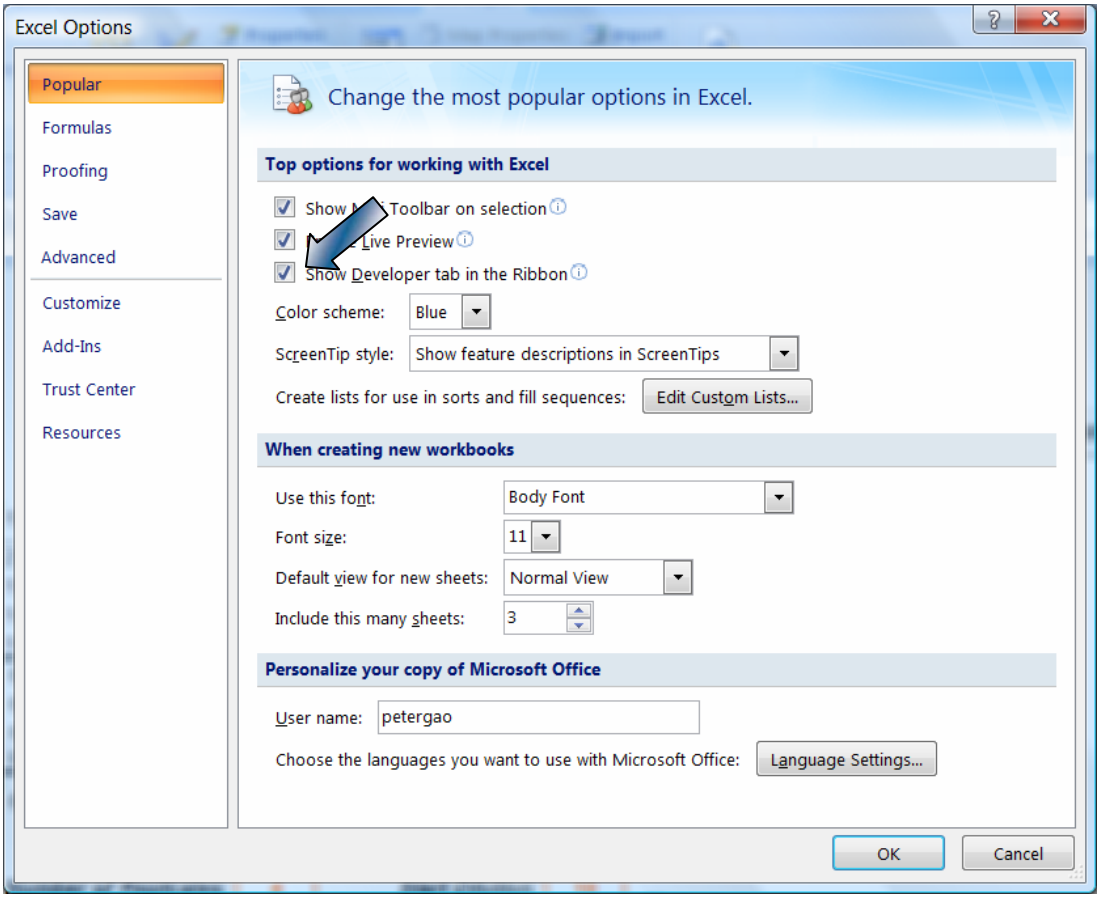



Options
View
Calculation
Edit
General
Transition
Custom Lists
Chart
Color
International
Save
Error Checking
Spelling
Security
File encryption settings for this workbook
Password to open:
Advanced...
File sharing settings for this workbook
Password to modify:
Read-only recommended
Digital Signatures...
Privacy options
Remove personal information from file properties on save
Macro security
Adjust the security level for files that might contain macro viruses and specify names of trusted macro developers.
Macro Security...
OK
Cancel

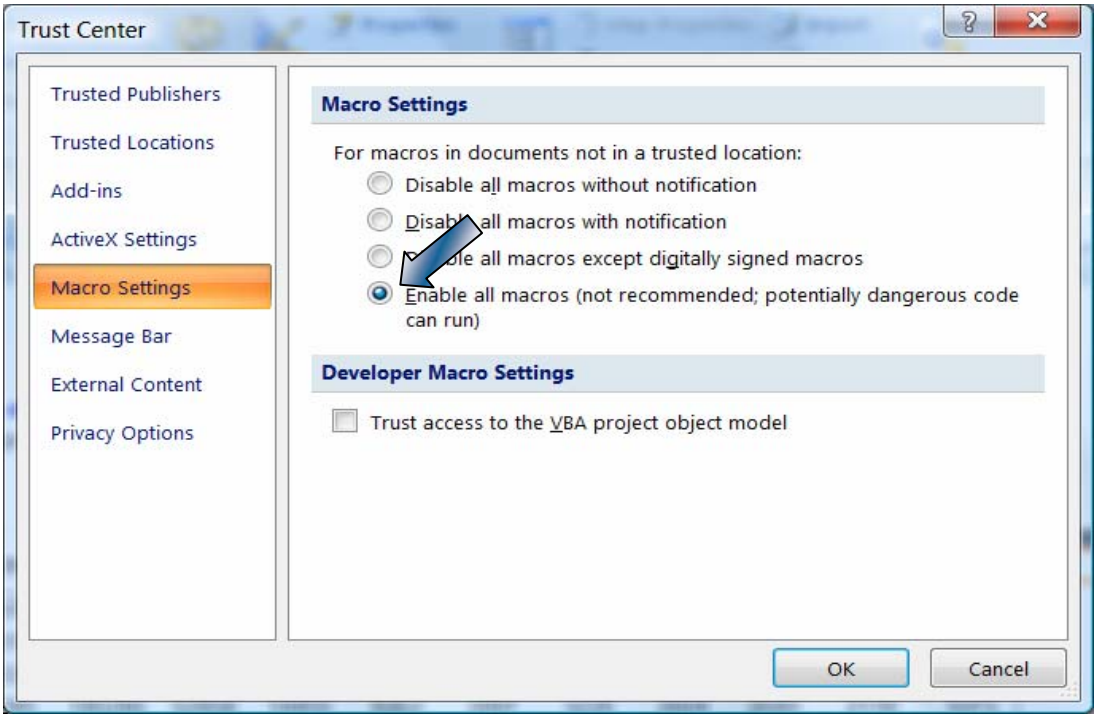

Security
Security Level
Trusted Publishers
Very High. Only macros installed in trusted locations will be allowed to run. All other signed and unsigned macros are disabled.
High. Only signed macros from trusted sources will be allowed to run. Unsigned macros are automatically disabled.
Medium. You can choose whether or not to run potentially unsafe macros.
Low (not recommended). You are not protected from potentially unsafe macros. Use this setting only if you have virus scanning software installed, or you have checked the safety of all documents you open.
OK
Cancel

For Window Vista Users:,

1. Add the Developer tab to Excel from Excel option
2. Check the Show Developer tab in the Ribbon, and click OK button
3. From the developer tab, click Macro Security



With the Trusted Center, check Enable all macros (not recommended; potentially dangerous code can run) , then click the OK button.



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

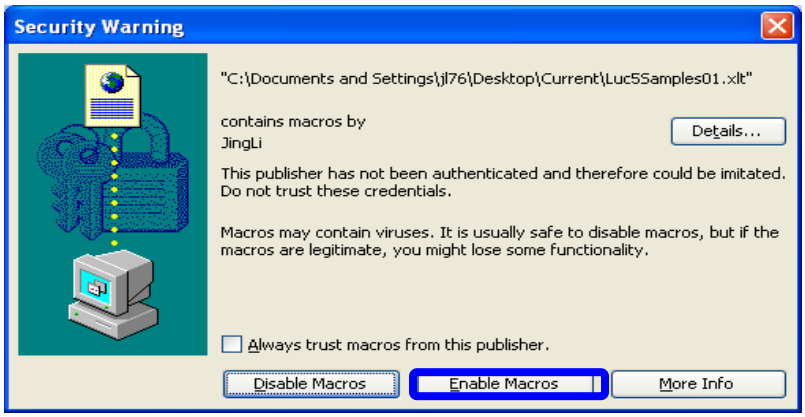
After this configuration is completed, it will not be necessary to change the macro security settings each time the macro is used.

III. Step by Step (Window XP)

1. Import the macro file entitled **"Luc5Samples04.xlt"**

2. Double click to open this file.

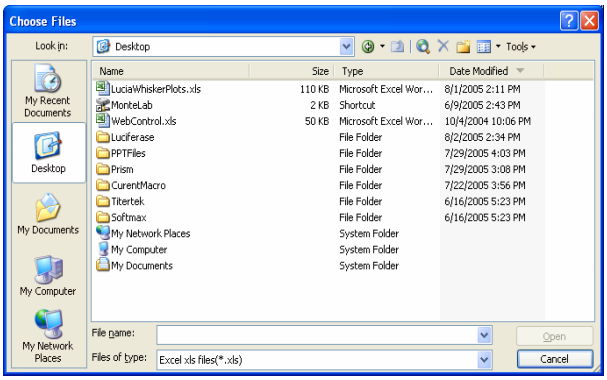
3. Click **"Enable Macros".**



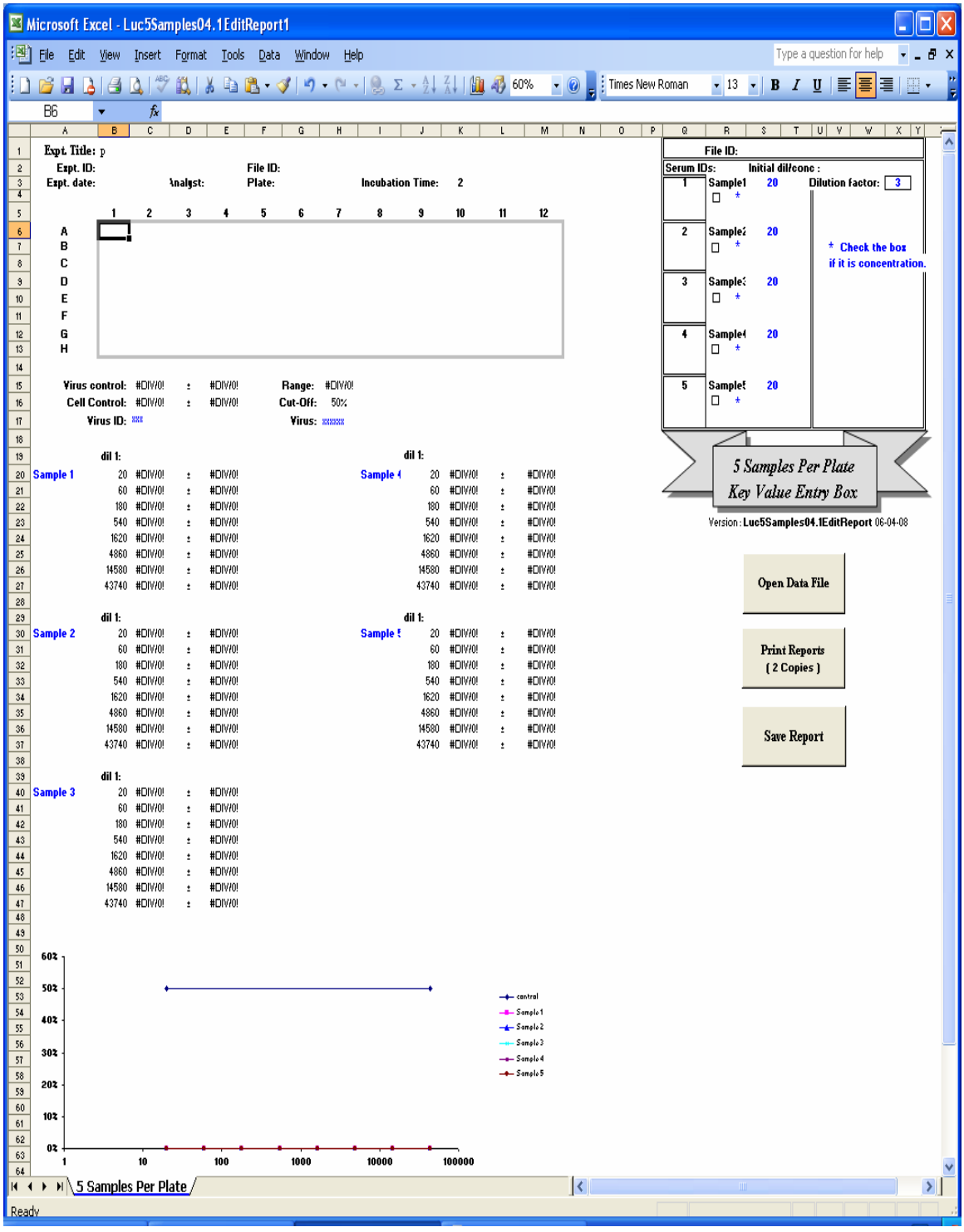
After the macro file is opened, the data area is blank waiting for data input.

4. Click **"Open Data File"** button to read data file.

Users can either open a raw data file generated by luminometer or a report file which was previously generated.

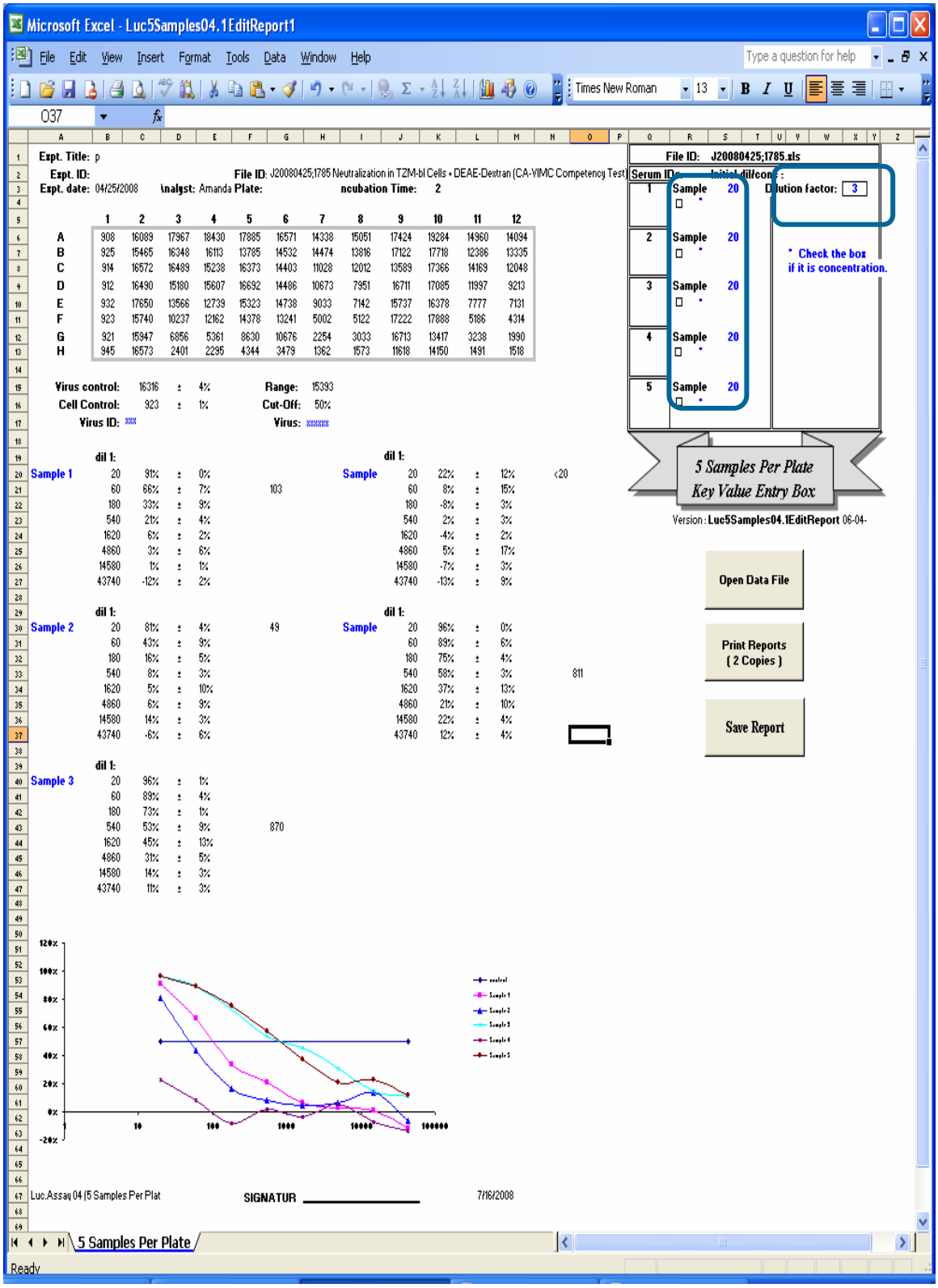


5. Click the data file name and then click Open button to open the data file.

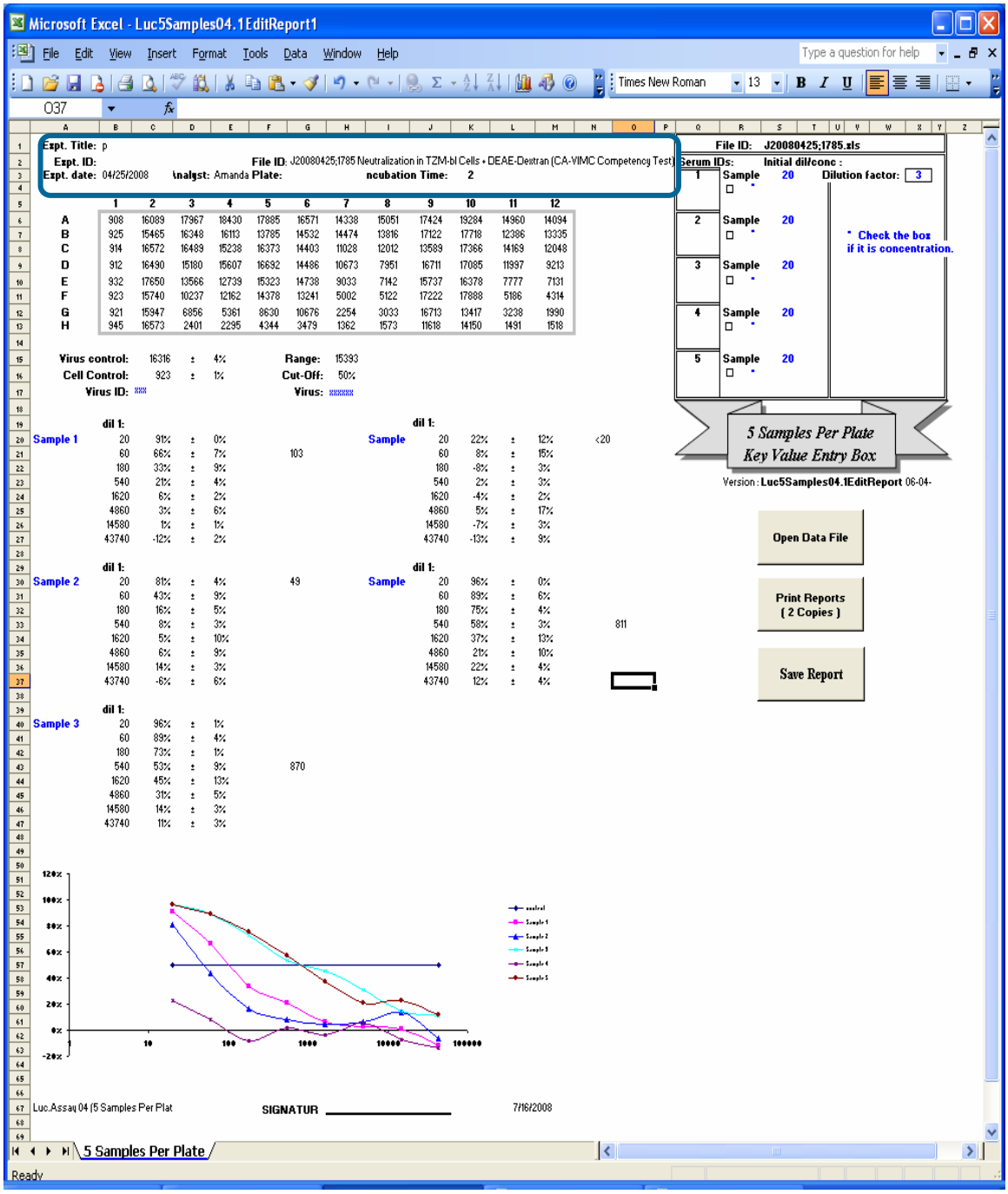


6. Enter appropriate sample **starting dilutions** (or **concentrations**) and **dilution factor**.

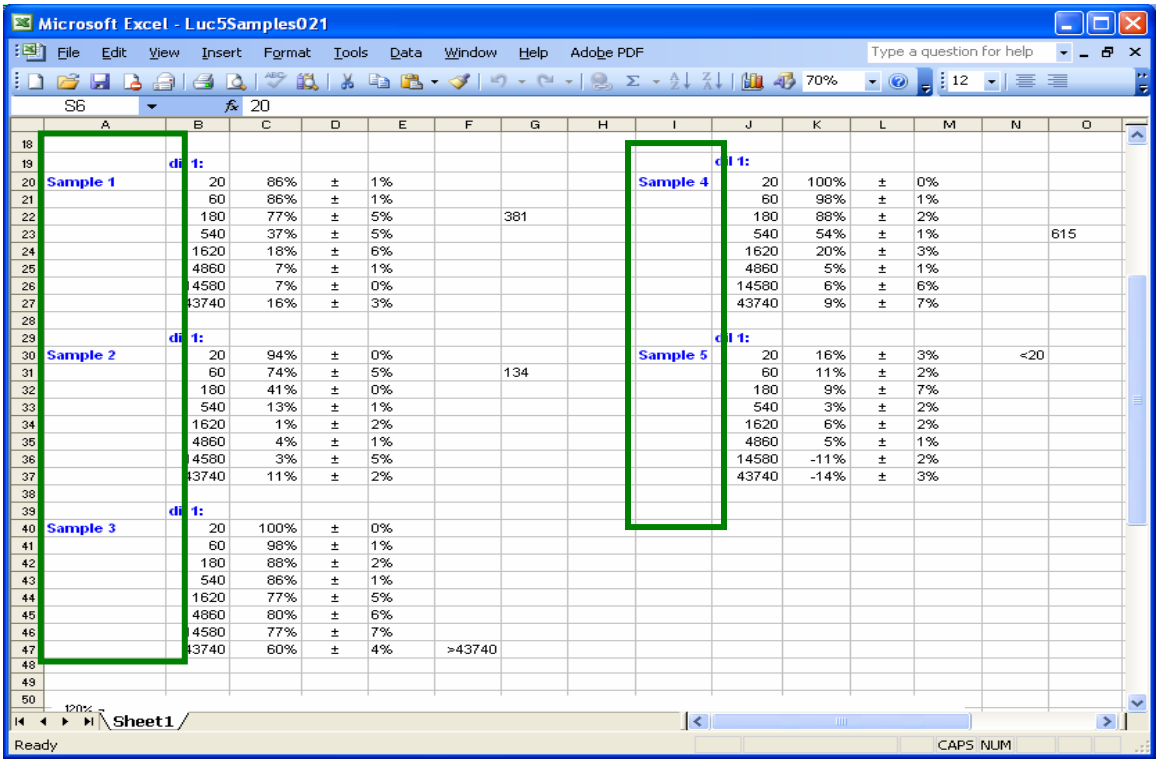
Enter the starting dilution, concentration code (if required) and dilution factor directly as indicated below.


Microsoft Excel - Luc5Samples04.1EditReport1
Expt. Title: p
Expt. ID:
Expt. date: 04/25/2008
Analyst: Amanda
Plate:
Incubation Time: 2
File ID: J20080425;1785 Neutralization in TZM-bl Cells + DEAE-Dextran (CA-VIMC Competency Test)
Virus control: 16316 ± 4%
Cell Control: 923 ± 1%
Range: 15393
Cut-Off: 50%
Virus ID:
Virus:
Sample 1
Sample 2
Sample 3
Sample
File ID: J20080425;1785.xls
Dilution factor: 3
* Check the box if it is concentration.
5 Samples Per Plate Key Value Entry Box
Version: Luc5Samples04.1EditReport 06-04-
Open Data File
Print Reports (2 Copies)
Save Report
SIGNATUR
7/16/2008
5 Samples Per Plate
Ready

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



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



9. Print Reports (two copies).

Click this button and two copies of the data report will be printed; one for the Principal Investigator and the other one will be kept by the technician.

11. Click “Save Report” button to save the report.

Click this button to save the report of your assay data.

Help

Please feel free to contact Peter Gao (*juanfei.gao@duke.edu*) if you have any questions about this guide or encounter any problems. Any suggestions for improvement are also welcome.