

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.

The screenshot displays the Microsoft Excel interface for a macro report titled "Luc5Samples04.1 EditReport1". The spreadsheet is organized into several sections:

- Header Section (Rows 1-5):** Contains fields for "Expt. Title: p", "Expt. ID:", "Expt. date:", "Analgst:", "File ID:", "Plate:", and "Incubation Time: 2".
- Sample Data Tables (Rows 20-47):** Three tables labeled "Sample 1", "Sample 2", and "Sample 3". Each table lists dilutions (20, 60, 180, 540, 1620, 4860, 14580, 43740) and corresponding values for "#DIV/0!".
- Graph (Rows 51-64):** A line graph with a logarithmic x-axis (1 to 100,000) and a linear y-axis (0% to 60%). The legend includes "control", "Sample 1", "Sample 2", "Sample 3", "Sample 4", and "Sample 5". A blue arrow points to the 50% mark on the y-axis.
- Right Sidebar (Rows 25-61):** A "5 Samples Per Plate Key Value Entry Box" containing:
 - File ID: []
 - Serum IDs: 1-5, each with "Sample:", "Initial dil/conc:", and "Dilution factor:" fields.
 - Buttons: "Open Data File", "Print Reports (2 Copies)", "Save Report".
 - Version: Luc5Samples04.1 EditReport 06-04-08

Below is the screenshot after a data file is imported.

Title

Expt. Title: p
 Expt. ID: File ID: J20080425;1785 Neutralization in TZM-bl Cells - DEAE-Dextran (CA-VIMC Competency Tes
 Expt. date: 04/25/2008 Inalgst: Amanda Plate: incubation Time: 2

Raw luminescence values imported from data file.

	1	2	3	4	5	6	7	8	9	10	11	12
908	16089	17967	18430	17885	16571	14338	15051	17424	19284	14960	14094	
925	15465	16348	16113	13785	14532	14474	13816	17122	17718	12386	13335	
914	16572	16489	15238	16373	14403	11028	12012	13589	17366	14169	12048	
912	16490	15180	15607	16692	14486	10673	7951	16711	17085	11987	9213	
932	17650	13666	12739	15323	14738	9033	7142	16737	16378	7777	7131	
923	16740	10237	12162	14378	13241	5002	5122	17222	17888	5186	4314	
921	16947	6856	5361	8630	10676	2254	3033	16713	13417	3238	1990	
945	16573	2401	2295	4344	3479	1362	1573	11618	14150	1491	1518	

Main data area

Virus control: 16316 ± 4% Range: 15393
 Cell Control: 923 ± 1% Cut-Off: 50%
 Virus ID: [REDACTED]

Sample 1

20	91% ± 0%		Sample	20	22% ± 12%
60	66% ± 7%	103		60	8% ± 15%
180	33% ± 9%			180	-8% ± 3%
540	21% ± 4%			540	2% ± 3%
1620	8% ± 2%			1620	-4% ± 2%
4860	3% ± 6%			4860	5% ± 17%
14580	1% ± 1%			14580	-7% ± 3%
43740	-12% ± 2%			43740	-13% ± 9%

Sample 2

20	91% ± 4%	49	Sample	20	96% ± 0%
60	43% ± 9%			60	89% ± 6%
180	16% ± 5%			180	75% ± 4%
540	8% ± 3%			540	58% ± 3%
1620	5% ± 10%			1620	37% ± 13%
4860	8% ± 9%			4860	21% ± 10%
14580	14% ± 3%			14580	22% ± 4%
43740	-6% ± 6%			43740	12% ± 4%

Sample 3

20	96% ± 1%	870			
60	89% ± 4%				
180	73% ± 1%				
540	53% ± 9%				
1620	45% ± 13%				
4860	31% ± 5%				
14580	14% ± 3%				
43740	11% ± 3%				

Graph Area

Luc.Assay 04 (5 Samples Per Plat) SIGNATUR _____ 7/16/2008

5 Samples Per Plate

Starting concentration/dilution, sample dilution factor, and appropriate code for concentration or dilution should be entered in this box.

Raw luminescence values imported from data file.

Main data area

5 Samples Per Plate
 Key Value Entry Box

Open Data File

Print Reports (2 Copies)

Save Report

Version of macro information

Data Import Button

Print Data Reports button

Save Data Reports Button

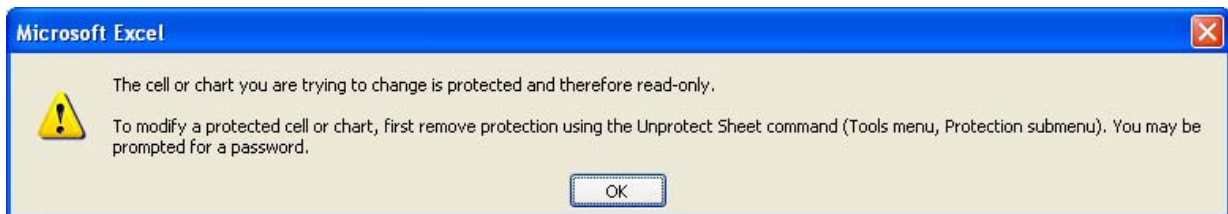
Information for assay name, user signature, and current date

Security Feature

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

The screenshot displays a Microsoft Excel spreadsheet titled 'Luc5Samples04.1EditReport1'. The main data area contains a table with columns labeled A through H and rows 1 through 12. Below the table, there are summary statistics for 'Virus control', 'Cell Control', and 'Virus ID'. A '5 Samples Per Plate Key Value Entry Box' is overlaid on the right side of the spreadsheet, containing a 'Serum ID' field, a 'Dilution factor' field, and a 'Check the box if it is concentration' checkbox. Below the entry box are three buttons: 'Open Data File', 'Print Reports [2 Copies]', and 'Save Report'. At the bottom of the spreadsheet, there is a line graph showing data for five samples (Sample 1 to Sample 5) across a range of values from 10 to 100,000. The graph shows a general downward trend for all samples as the x-axis value increases.

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

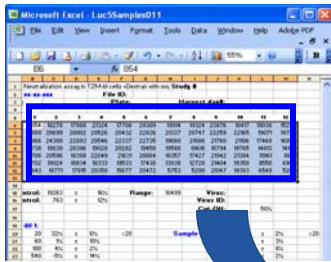
The templates for 5 samples per plate neutralization assays

	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

Plate Set-up

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



	1	2	3	4	5	6	7	8	9	10	11	12
A	701	67373	56971	54080	58049	60151	24908	28396	56761	63676	74423	76828
B	67373	61825	61389	61825	62049	66216	19008	12554	59008	64976	72156	74302
C	61825	60885	61925	60885	64109	63644	16558	11254	62190	63416	62485	63524
D	60885	614	57116	1900	66090	64436	18054	3211	51413	4580	63716	11460
E	614	68589	44230	9156	58249	7212	9800	0507	31142	0463	65418	3548
F	68589	63243	18054	3211	39049	9472	9188	7759	9188	7759	56761	3676
G	63243	10721	9344	15334	19631	1970	1488	1970	1488	1970	58049	60151
H	10721	66471	9800	10507	4346	4808	1002	746	1002	746	58971	54080

(In the program file)

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

19		dil 1:							
20	Sample 1	20	86%	±	1%				
21		60	86%	±	1%		381		
22		180	77%	±	5%				
23		540	37%	±	5%				
24		1620	18%	±	6%				615
25		4860	7%	±	1%				
26		14580	7%	±	0%				
27		43740	16%	±	3%				
28									
29		dil 1:							
30	Sample 2	20	94%	±	0%				
31		60	74%	±	5%		134		
32		180	41%	±	0%				
33		540	13%	±	1%				
34		1620	1%	±	2%				
35		4860	1%	±	1%				
36		14580	3%	±	5%				
37		43740	11%	±	2%				
38									
39		dil 1:							
40	Sample 3	20	100%	±	0%				
41		60	98%	±	1%				
42		180	88%	±	2%				
43		540	88%	±	1%				
44		1620	5%	±	5%				
45		4860	80%	±	6%				
46		14580	77%	±	7%				
47		43740	60%	±	4%		>43740		
48									
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Let's take sample 1 as an example. From the top to bottom are dilutions 1 - 8.

19		dil 1:							
20	Sample 1	20	86%	±	1%			Dilution 1= 1:20	
21		60	86%	±	1%			Dilution 2= 1:60	
22		180	77%	±	5%			Dilution 3= 1:180	
23		540	37%	±	5%			Dilution 4= 1:540	
24		1620	18%	±	6%			Dilution 5= 1:1620	
25		4860	7%	±	1%			Dilution 6= 1:4860	
26		14580	7%	±	0%			Dilution 7= 1:14580	
27		43740	16%	±	3%			Dilution 8= 1:43740	
28									
29									
30									
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100									

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															
49															

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 Xmmddy\$\$;####.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 “Xmmddy\$\$;####.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 “Xmmddy\$\$;####.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.DPB-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.

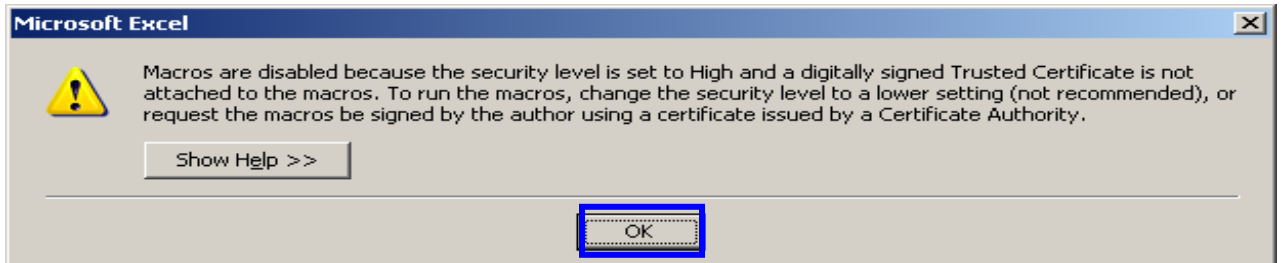
Note: If the name of the report does not include “report”, the macro can not open the report automatically.

Please note that the raw and analyzed data section is still locked and cannot be modified!

II. Configuration

Macro Security Setting Change

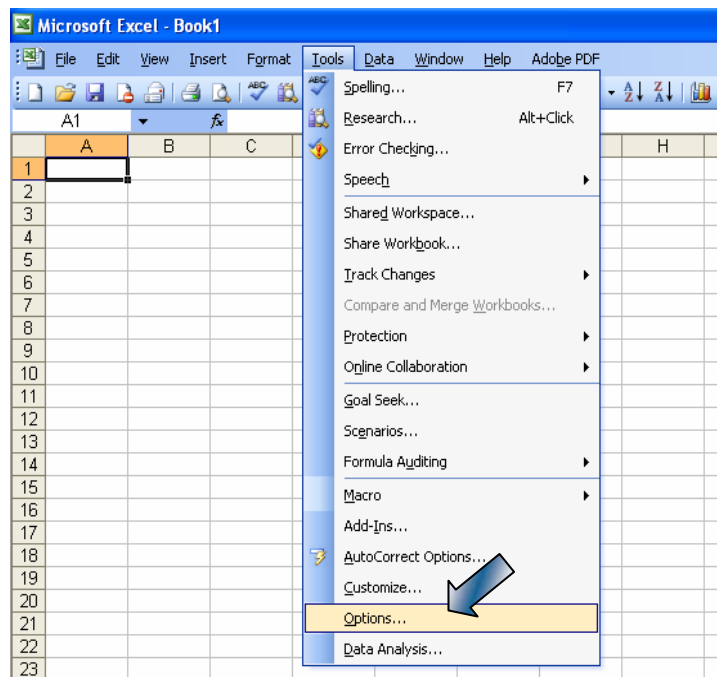
The first time the luminescence macro program is opened, the following dialog box may appear, prompting the operator to change the Excel Macro security settings.

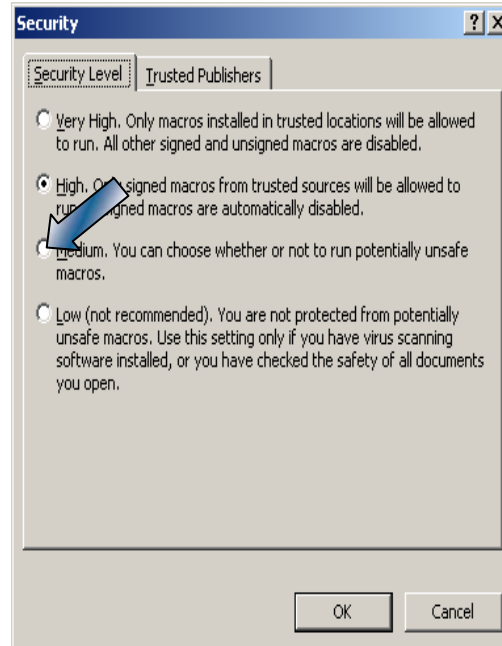
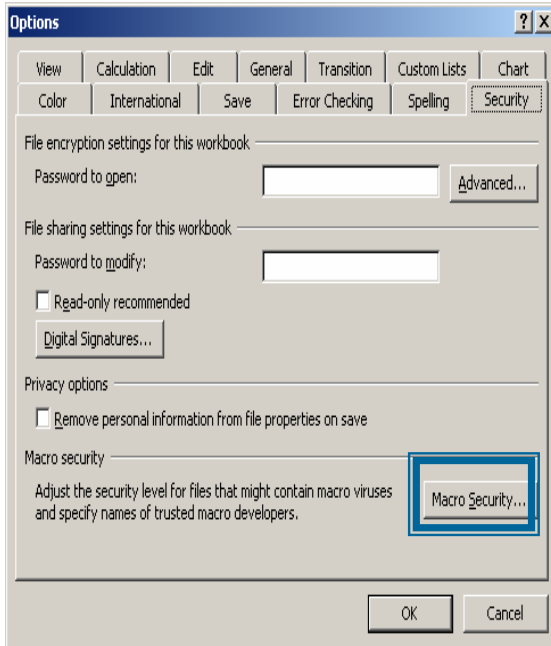


Windows XP Users:

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

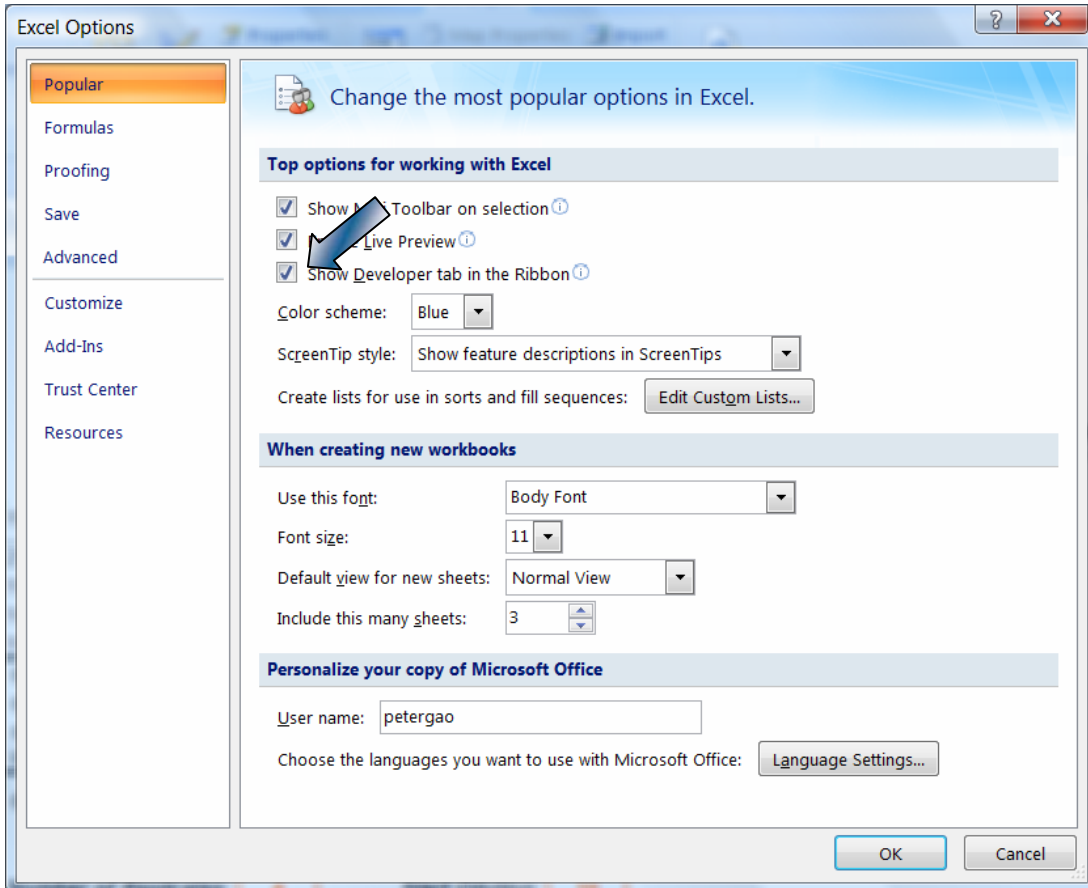
1. Click **OK** button in the above dialog box.
2. On the **Tools** menu, click **Options**.
3. Click the **Security** tab.
4. Under **Macro Security**, click **Macro Security**.
5. Click the **Security Level** tab, and select **Medium**.
6. Click **Ok** to close the dialog boxes.



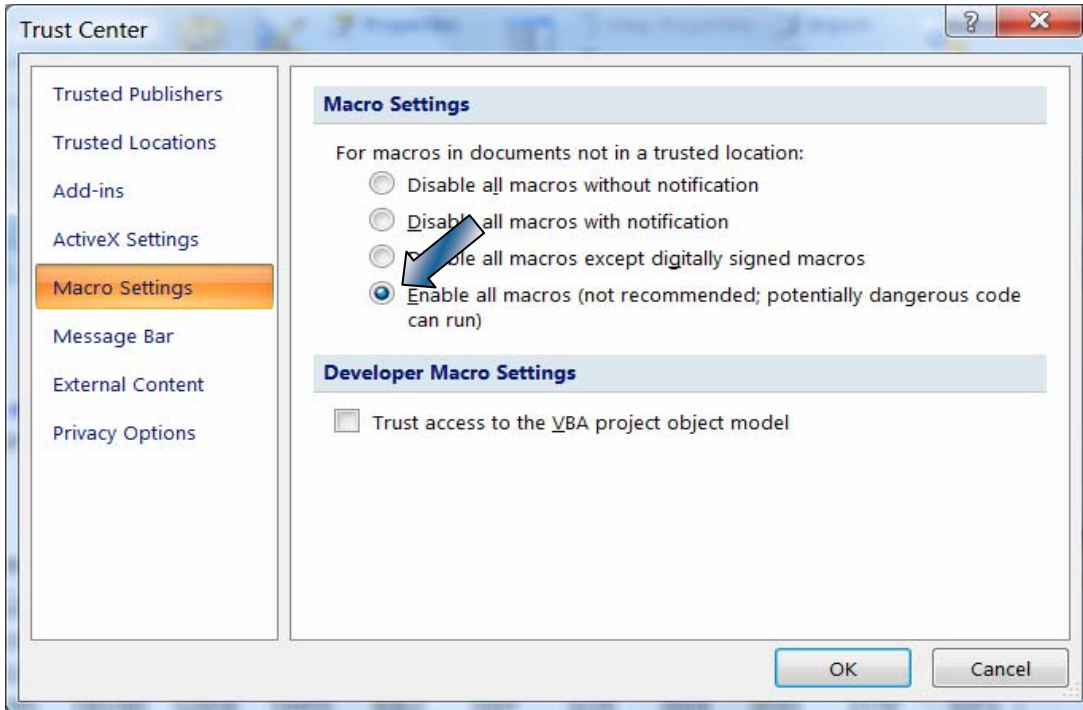


For Window Vista Users:

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.

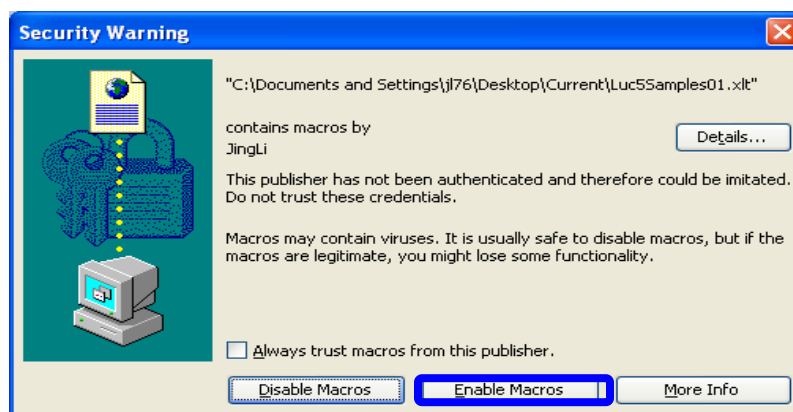
After this configuration is completed, it will not be necessary to change the macro security settings each time the macro is used.

III. Step by Step (Window XP)

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

2. Double click to open this file.

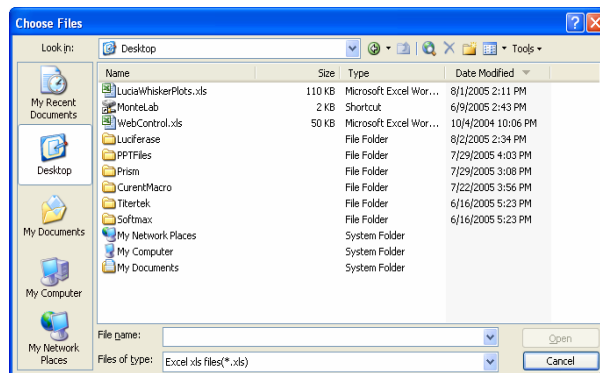
3. Click **“Enable Macros”**.



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.

The screenshot displays a Microsoft Excel spreadsheet titled "Luc5Samples04.1EditReport1". The spreadsheet is divided into several sections:

- Header Section (Rows 1-5):** Contains fields for "Expt. Title: p", "Expt. ID:", "Expt. date:", "Analyst:", "File ID:", "Plate:", and "Incubation Time: 2".
- Grid Section (Rows 6-14):** A grid with columns labeled 1-12 and rows labeled A-H.
- Control Section (Rows 15-17):** Fields for "Virus control: #DIV/0!", "Cell Control: #DIV/0!", "Virus ID: xxx", "Range: #DIV/0!", "Cut-Off: 50%", and "Virus: #DIV/0!".
- Sample Entry Section (Rows 19-38):** A table for entering sample data. It is organized into three columns for "Sample 1", "Sample 2", and "Sample 3". Each column has a "dil 1:" header and five rows of data points (20, 60, 180, 540, 1620, 4860, 14580, 43740) with corresponding "#DIV/0!" values.
- Key Value Entry Box (Rows 39-40):** A box titled "5 Samples Per Plate Key Value Entry Box" containing a table for "Serum IDs" and "Initial dil/conc". The table has 5 rows, each with "Sample", "Initial dil/conc" (value 20), and "Dilution factor" (value 3). A note says "+ Check the box if it is concentration." Below the table is a "Version: Luc5Samples04.1EditReport 06-04-08" label.
- Buttons (Rows 41-43):** Three buttons: "Open Data File", "Print Reports (2 Copies)", and "Save Report".
- Graph (Rows 50-64):** A log-log plot with the y-axis ranging from 0 to 602 and the x-axis ranging from 1 to 100000. The legend includes "control", "Sample 1", "Sample 2", "Sample 3", "Sample 4", and "Sample 5". A horizontal line is drawn at approximately y=500.

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

File Edit View Insert Format Tools Data Window Help

Type a question for help

O37

Expt. Title: p
 Expt. ID: File ID: J20080425.1785 Neutralization in TZM-bl Cells + DEAE-Dextran (CA-VIMC Competency Test)
 Expt. date: 04/25/2008 Inalyst: Amanda Plate: Incubation Time: 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	908	16089	17967	18430	17885	16571	14338	15051	17424	19284	14960	14094
B	925	16465	16348	16113	13785	14532	14474	13816	17122	17718	12386	13335
C	914	16572	16489	15238	16373	14403	11028	12012	13589	17366	14169	12048
D	912	16490	15180	15607	16692	14486	10673	7951	16711	17085	19397	9213
E	932	17650	13666	12739	15323	14738	9033	7142	16737	16378	7777	7131
F	923	16740	10237	12162	14378	13241	5002	5122	17222	17888	5186	4314
G	921	16947	6856	5361	8630	10676	2254	3033	16713	13417	3238	1990
H	945	16573	2401	2295	4344	3479	1362	1573	11618	14150	1491	1518

Virus control: 16316 ± 4% Range: 15393
 Cell Control: 923 ± 1% Cut-Off: 50%
 Virus ID: Virus:

Sample	dil 1:	91%	± 0%	103	Sample	dil 1:	20	22%	± 12%
Sample 1	20	91%	± 0%	103	Sample	20	22%	± 12%	<20
	60	86%	± 7%			60	8%	± 15%	
	180	33%	± 9%			180	-8%	± 3%	
	540	21%	± 4%			540	2%	± 3%	
	1620	6%	± 2%			1620	-4%	± 2%	
	4860	3%	± 6%			4860	5%	± 17%	
	14580	1%	± 1%			14580	-7%	± 3%	
	43740	-12%	± 2%			43740	-13%	± 9%	

Sample	dil 1:	91%	± 4%	49	Sample	dil 1:	20	96%	± 0%
Sample 2	20	91%	± 4%	49	Sample	20	96%	± 0%	
	60	43%	± 9%			60	89%	± 6%	
	180	16%	± 5%			180	75%	± 4%	
	540	8%	± 3%			540	58%	± 3%	
	1620	5%	± 10%			1620	37%	± 13%	
	4860	6%	± 9%			4860	21%	± 10%	
	14580	14%	± 3%			14580	22%	± 4%	
	43740	-6%	± 6%			43740	12%	± 4%	

Sample	dil 1:	20	96%	± 1%	870
Sample 3	20	96%	± 1%	870	
	60	89%	± 4%		
	180	73%	± 1%		
	540	53%	± 9%		
	1620	45%	± 13%		
	4860	31%	± 5%		
	14580	14%	± 3%		
	43740	11%	± 3%		

5 Samples Per Plate
Key Value Entry Box

Version: Luc5Samples04.1EditReport 06-04

Open Data File
 Print Reports (2 Copies)
 Save Report

Luc Assay 04 (5 Samples Per Plat) SIGNATUR _____ 7/16/2008

5 Samples Per Plate

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

The screenshot displays an Excel spreadsheet with the following data tables:

	1	2	3	4	5	6	7	8	9	10	11	12
A	908	16089	17967	18430	17885	16571	14338	16051	17424	19284	14960	14094
B	925	15465	16348	16103	13785	14532	14474	13816	17122	17718	12386	13335
C	914	16572	16489	15238	16373	14403	11028	12012	13589	17366	14169	12048
D	912	16490	15180	15607	16692	14486	10673	7951	16711	17085	11997	9213
E	932	17650	13566	12739	15323	14738	9033	7142	16737	16378	7777	7131
F	923	15740	10237	12162	14378	13241	5002	5122	17222	17888	5186	4314
G	921	15947	6856	5361	8630	10676	2254	3033	16713	13417	3238	1990
H	945	16573	2401	2295	4344	3479	1362	1573	11618	14150	1491	1518

Sample	Dilution	Dilution factor
1	Sample 20	3
2	Sample 20	
3	Sample 20	
4	Sample 20	
5	Sample 20	

Sample	dil 1:	%	±	Value
Sample 1	20	91%	± 0%	
	60	66%	± 7%	103
	180	33%	± 9%	
	540	21%	± 4%	
	1620	8%	± 2%	
Sample 2	20	91%	± 4%	49
	60	43%	± 9%	
	180	16%	± 5%	
	540	8%	± 3%	
	1620	5%	± 10%	
Sample 3	20	96%	± 1%	870
	60	89%	± 4%	
	180	73%	± 1%	
	540	53%	± 9%	
	1620	45%	± 13%	

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

Sample	Dilution	Value	%	±	Value	%	±	Value	%	±
Sample 1	dil 1:	20	86%	±	1%					
		60	86%	±	1%					
		180	77%	±	5%	381				
		540	37%	±	5%					615
		1620	18%	±	6%					
Sample 2	dil 1:	20	94%	±	0%					
		60	74%	±	5%	134				
		180	41%	±	0%					
		540	13%	±	1%					
		1620	1%	±	2%					
Sample 3	dil 1:	20	100%	±	0%					
		60	98%	±	1%					
		180	88%	±	2%					
		540	86%	±	1%					
		1620	77%	±	5%					
Sample 4	dil 1:	20	100%	±	0%					
		60	98%	±	1%					
		180	88%	±	2%					
		540	54%	±	1%					
		1620	20%	±	3%					
Sample 5	dil 1:	20	16%	±	3%					<20
		60	11%	±	2%					
		180	9%	±	7%					
		540	3%	±	2%					
		1620	6%	±	2%					

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.