

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.

The screenshot displays the Microsoft Excel interface for a macro file named "Luc5Samples041". The spreadsheet is organized as follows:

- Header Section (Rows 1-4):**
 - Row 1: **Expt. Title:** Neutralization assay in TZM-bl cells +Dextran with xxxx; **Study #**
 - Row 2: **Expt. ID:** xx-xx-xxx
 - Row 3: **Expt. date:** [blank]
 - Row 4: **File ID:** [blank], **Plate:** [blank], **Incubation Time:** 2
- Grid Section (Rows 6-14, Columns 1-12):** A large empty grid for data entry, with columns labeled 1 through 12 and rows labeled A through H.
- Control and Sample Data (Rows 15-38):**
 - Row 15: **Virus control:** #DIV/0! ; #DIV/0! **Range:** #DIV/0!
 - Row 16: **Cell Control:** #DIV/0! ; #DIV/0! **Cut-Off:** 50%
 - Row 17: **Virus ID:** xxx **Virus:** xxxxxxx
 - Rows 19-27: **Sample 1** and **Sample 4** data tables with columns for dilution (20, 60, 180, 540, 1620, 4860, 14580, 43740) and corresponding values.
 - Rows 30-37: **Sample 2** and **Sample 5** data tables with the same structure as Sample 1 and 4.
 - Rows 40-47: **Sample 3** data table with the same structure.
- Graph (Rows 50-63):** A line graph showing luminescence data. The y-axis ranges from 0% to 60%. A horizontal line at 50% is labeled "control". Data points for Sample 1 through Sample 5 are plotted near the 0% mark. A legend identifies the series: control (blue), Sample 1 (magenta), Sample 2 (cyan), Sample 3 (green), Sample 4 (red), and Sample 5 (purple).
- Right Sidebar (Rows 250-450):**
 - File ID:** [blank]
 - Serum IDs:** 1-5, each with a "Sample" checkbox and a "20" value.
 - Initial dil/conc:** [blank]
 - Dilution factor:** 3
 - Instruction:** * Check the box if it is concentration.
 - Key Value Entry Box:** 5 Samples Per Plate
 - Buttons:** Get Luminescence Data, Print Reports (2 Copies), Save Reports To the Server.

Below is the screenshot after a data file is imported.

Microsoft Excel - Luc5Samples041

File Edit View Insert Format Tools Data Window Help Adobe PDF

WV58 fx

Title

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.

	1	2	3	4	5	6	7	8	9	10	11	12
A	779	30693	28809	30839	18299	19273	24998	27931	26460	31619	29020	37094
B	736	26843	28486	27377	17097	17126	21370	23224	28241	28163	28166	30724
C	699	29402	29581	28313	17251	17173	19428	20679	28668	32578	29359	28429
D	656	27020	29383	25863	14538	14423	15921	18567	28798	25847	27366	29124
E	619	23567	28261	27229	15062	12839	12840	14299	28934	29321	32625	29905
F	620	22634	27195	29541	7254	7673	8284	9804	28043	31357	29782	31339
G	589	22873	26401	26000	1237	1319	2467	2408	29939	31428	34178	34360
H	697	25378	10697	10272	281	226	593	657	25213	25976	40263	42765

Expt. Title: Neutralization assay in T2M-bl cells +Dextran with xxx; **Study #**
Expt. ID: xx-xx-xxx **File ID:** E20050818;3932 P787 T2M-bl cells,
Expt. date: 08/18/2005 **Hongmei Plate:** **Incubation Time:** 2

5 Samples Per Plate Key Value Entry Box
 Serum ID: E20050818;3932 P787 T2M-bl cells
 Initial dil/conc: 20
 Dilution factor: 3
 * Check the box if it is concentration.

Experiment results

Sample	dil 1:	Value	%	±	Code
Sample 1	20	61%	±	1%	24
	60	-1%	±	1%	
	180	-9%	±	7%	
	540	-7%	±	3%	
	1620	-6%	±	10%	
Sample 2	20	100%	±	0%	
	60	98%	±	0%	
	180	73%	±	1%	489
	540	48%	±	6%	
	1620	46%	±	0%	
Sample 3	20	100%	±	0%	
	60	93%	±	0%	
	180	67%	±	4%	514
	540	49%	±	4%	
	1620	35%	±	7%	
Sample 4	20	2%	±	2%	<20
	60	-18%	±	4%	
	180	-14%	±	9%	
	540	-12%	±	1%	
	1620	-5%	±	8%	
Sample 5	20	-61%	±	7%	<20
	60	-32%	±	1%	
	180	-18%	±	4%	
	540	-21%	±	8%	
	1620	-9%	±	5%	

Graph Area

control
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5

120%
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%

10 100 1000 10000 100000

Get Luminescence Data
Print Reports (2 Copies)
Save Reports To the Server

Data Import Button
Print Data Reports
Save E-Copy of this report to the file server

Security Feature

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

The screenshot displays a Microsoft Excel spreadsheet titled "Luc55Samples041". The main data table (rows 6-13, columns 1-12) contains numerical values for samples A through H. Below this, there are sections for "Virus control", "Cell Control", and "Virus ID". A graph at the bottom shows luminescence on the y-axis (ranging from -40% to 120%) against dilution on the x-axis (log scale from 10 to 10000). The graph includes data for a control and five samples. A dialog box titled "5 Samples Per Plate Key Value Entry Box" is open on the right, with fields for "Sample", "Initial dilution", and "Dilution factor". The dialog box also contains a note: "+ Check the box if it is concentration." and buttons for "Get Luminescence Data", "Print Reports (2 Copies)", and "Save Reports To the Server".

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

The screenshot shows a Microsoft Excel error dialog box with a yellow warning icon. The text inside the dialog box reads: "The cell or chart you are trying to change is protected and therefore read-only. To modify a protected cell or chart, first remove protection using the Unprotect Sheet command (Tools menu, Protection submenu). You may be prompted for a password." An "OK" button is located at the bottom center of the dialog box.

Template of 5 Samples per Plate

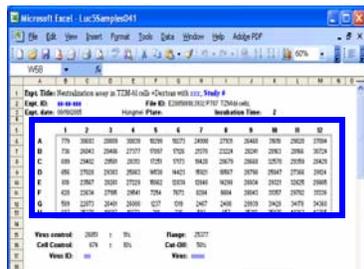
The templates for 5 samples per plate neutralization assays

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

Sample 1
Sample 2
Sample 3
Sample 4
Sample 5

Plate set-up in the protocol

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	61389	61825	62040	66216	19008	12554	59008	64976	72156	74302	
C	67373	61926	60885	64109	63644	16558	11254	62190	63416	62485	63524	
D	67373	57116	61900	66090	64436	18054	13211	51413	4580	63716	61460	
E	67373	68589	44230	39156	58249	7212	9800	10507	31142	10463	65418	63548
F	67373	18054	3211	39049	9472	9188	7759	9188	7759	56761	63676	
G	67373	10721	9344	15334	19631	1970	1488	1970	1488	58049	60151	
H	678	66471	9800	10507	4346	4808	1002	746	1002	746	56971	54080

(In the program file)

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

18					
19	dil 1:				
20	Sample 1	20	61%	± 1%	24
21		60	-1%	± 1%	
22		180	-9%	± 7%	
23		540	-7%	± 3%	
24		1620	-6%	± 10%	
25		4860	-11%	± 4%	
26		14580	-7%	± 3%	
27		43740	-15%	± 6%	
28					
29	dil 1:				
30	Sample 2	20	100%	± 0%	
31		60	98%	± 0%	
32		180	73%	± 1%	489
33		540	48%	± 6%	
34		1620	1%	± 0%	
35		4860	35%	± 0%	
36		14580	35%	± 0%	
37		43740	29%	± 3%	
38					
39	dil 1:				
40	Sample 3	20	100%	± 0%	
41		60	93%	± 0%	
42		180	67%	± 4%	514
43		540	1%	± 2%	
44		1620	3%	± 1%	
45		4860	24%	± 3%	
46		14580	15%	± 5%	
47		43740	-2%	± 8%	
48					
5 Samples Per Plate					
Ready					

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

19	dil 1:					
20	Sample 1	20	61%	±	1%	Dilution 1= 1:20
21		60	-1%	±	1%	Dilution 2= 1:60
22		180	-9%	±	7%	Dilution 3= 1:180
23		540	-7%	±	3%	Dilution 4= 1:540
24		1620	-6%	±	10%	Dilution 5= 1:1620
25		4860	-11%	±	4%	Dilution 6= 1:4860
26		14580	-7%	±	3%	Dilution 7= 1:14580
27		43740	-15%	±	6%	Dilution 8= 1:43740

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	61%	±	1%		24		Sample 4	20	2%	±	2%	<20	
21		60	-1%	±	1%					60	-18%	±	4%		
22		180	-9%	±	7%					180	-14%	±	9%		
23		540	-7%	±	3%					540	-12%	±	1%		
24		1620	-6%	±	10%					1620	-5%	±	8%		
25		4860	-11%	±	4%					4860	-18%	±	11%		
26		14580	-7%	±	3%					14580	-8%	±	0%		
27		43740	-15%	±	6%					43740	-12%	±	14%		
28															
29		dil 1:								dil 1:					
30	Sample 2	20	100%	±	0%				Sample 5	20	-61%	±	7%	<20	
31		60	98%	±	0%					60	-32%	±	1%		
32		180	73%	±	1%		489			180	-18%	±	4%		
33		540	48%	±	6%					540	-21%	±	8%		
34		1620	46%	±	0%					1620	-9%	±	5%		
35		4860	35%	±	0%					4860	-11%	±	3%		
36		14580	35%	±	0%					14580	-13%	±	7%		
37		43740	29%	±	3%					43740	-28%	±	22%		
38															
39		dil 1:								dil 1:					
40	Sample 3	20	100%	±	0%					20	-61%	±	7%	<20	
41		60	93%	±	0%					60	-32%	±	1%		
42		180	67%	±	4%		514			180	-18%	±	4%		
43		540	49%	±	4%					540	-21%	±	8%		
44		1620	35%	±	7%					1620	-9%	±	5%		
45		4860	24%	±	3%					4860	-11%	±	3%		
46		14580	15%	±	5%					14580	-13%	±	7%		
47		43740	-2%	±	8%					43740	-28%	±	22%		
48															

- 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

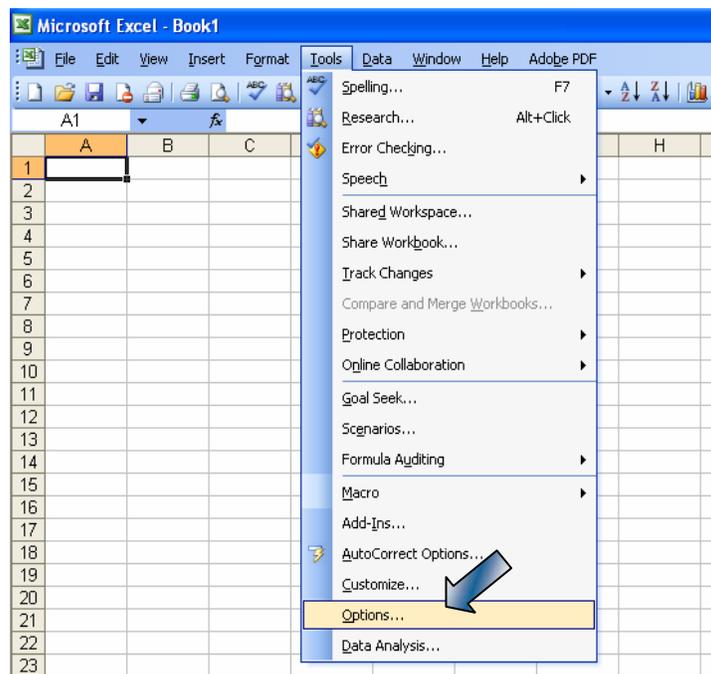
Macro Security Setting Change

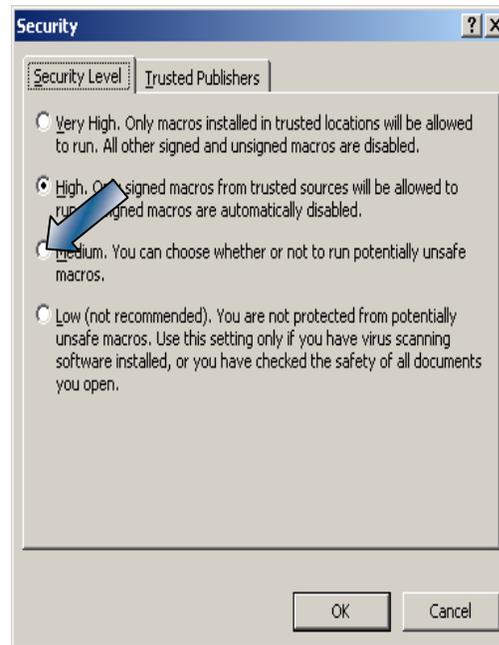
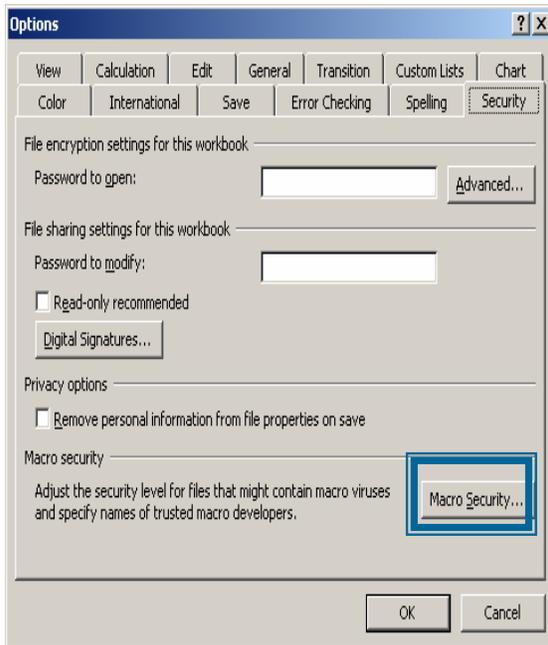
The first time you start the luminescence macro program, you may encounter the following dialog box, which prompts that you need to change Excel Macro security settings.



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.





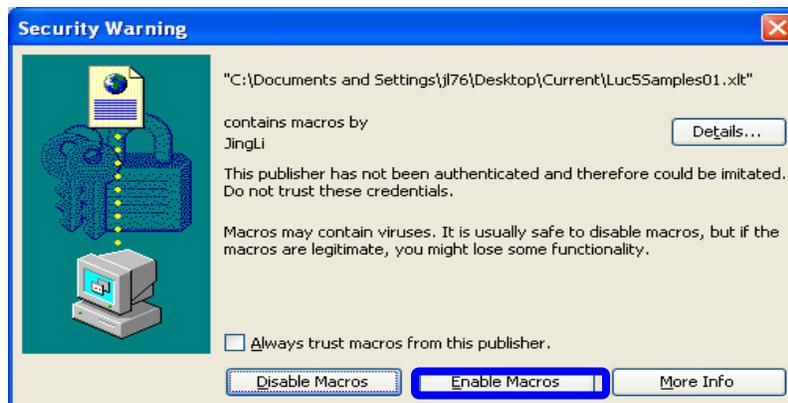
Close and then restart the Excel program. The disabled macros message should not appear.

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

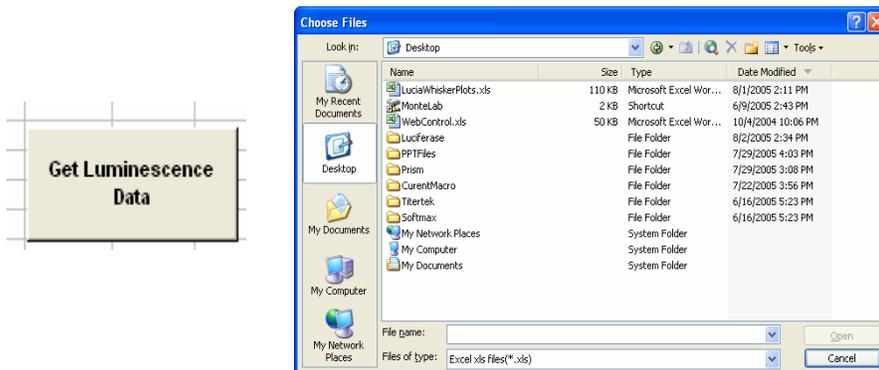
1. Open the macro file entitled “**Luc5Samples04.xlt**”.

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.

	P	Q	R	S	T	U	V	W	X	Y	
1		File ID: E20050818;3932 P787 T2M-bl cells.									
2		Serum IDs:		Initial dil/conc :							
3		1	Sample1	<input type="checkbox"/>	*	20	Dilution factor: <input type="text" value="3"/>				
4											
5											
6		2	Sample2	<input type="checkbox"/>	*	20	* Check the box if it is concentration.				
7											
8											
9		3	Sample3	<input type="checkbox"/>	*	20					
10											
11											
12		4	Sample4	<input type="checkbox"/>	*	20					
13											
14											
15		5	Sample5	<input type="checkbox"/>	*	20					
16											
17											
18											
19											
20											
21											
22											
23											

*5 Samples Per Plate
Key Value Entry Box*

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.*)

Sample	dil 1:	20	60	180	540	1620	4860	14580	43740
Sample 1		61%	-1%	-3%	-7%	-6%	-11%	-7%	-15%
Sample 2		100%	98%	73%	48%	46%	35%	35%	29%
Sample 3		100%	93%	67%	49%	35%	24%	15%	-2%
Sample 4		2%	-18%	-14%	-12%	-5%	-18%	-8%	-12%
Sample 5		-61%	-32%	-18%	-21%	-9%	-11%	-13%	-28%

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