

Quick Guide to TCID50 Macro

Below is the screenshot of the TCID50 macro.

TCID50 ASSAY

Expt. ID: XXXX-XX Virus Name: XXX
 Expt. Date: Virus Date: mm/dd/yy Date Assay Day: 2
 Performer: Assay Cells: TZM-bl

# pos	4	4	4	4	4	2	2	0	0	0	0	c ctrl.	bkg.
Mean	412962	173330	43204	10271	3757	1623	1382	1060	972	838	795	585	
A	469063	205981	53608	13614	5573	2129	1790	1343	861	831	744	587	
B	403958	160991	41197	9811	3535	1408	1562	1238	1026	843	669	570	
C	395363	161533	39291	9041	2943	1308	1060	762	1133	618	896	565	Virus 1
D	383464	164816	38720	8618	2977	1646	1115	898	867	1058	870	619	
E	182652	39084	11197	3003	1160	869	633	580	685	756	727	595	
F	172440	37251	7235	2480	870	745	708	626	813	678	601	666	
G	179808	34941	8801	2253	1021	754	882	772	746	617	613	649	
H	181549	32166	8326	2112	984	894	861	704	898	622	627	674	
Mean	179112	35861	8890	2462	1009	816	771	671	786	668	642	646	
# pos	4	4	4	4	0	0	0	0	0	0	0	c ctrl.	bkg.

Pos. Cutoff: 1463 Number of Replicates: 4 Start Dilution: 10
 Cutoff Factor: 2.50 Dilution Per ml: 5 Dilution Factor: 5

Dilution	# pos	# neg	pos & neg totals	% neg	Dilution	TCID50/Well
row 1	4	0	24	0	10	6988
2	4	0	20	0	50	1397.5
3	4	0	16	0	250	279.5
4	4	0	12	0	1250	55.902
5	4	0	8	0	6250	11.180
6	2	2	4	2	31250	2.236
7	2	2	2	4	156250	0.447
8	0	4	0	8	781250	0.0894
9	0	4	0	12	3906250	0.0179
10	0	4	0	16	19531250	0.0036
11	0	4	0	20	97656250	0.00072

(totals) 24 20
 Dilution at 50% is: 69877

TCID50/ml 349,386

Open File

RLU vs Dilution graph showing a sigmoidal curve with data points labeled: 6988, 1397.5, 279.5, 55.902, 11.180, 2.236, 0.447, 0.0894, 0.0179, 0.0036, 0.00072.

1. Click “Open File” button to the import the raw data file to the macro file.
2. If the text font color is orange, it means that this information can be changed by the user.
3. If the text font color is blue, it means that this information needs to be entered by the user.
4. The TCID50 value will be shown in the red box in red font.
5. The drop down list allows the user to choose which virus to analyze: Virus 1 or Virus 2. (See figure below)
6. If Virus 1 is selected, the top half of raw data box will be highlighted (see green box below) and Virus 1 will be shown next to the box.

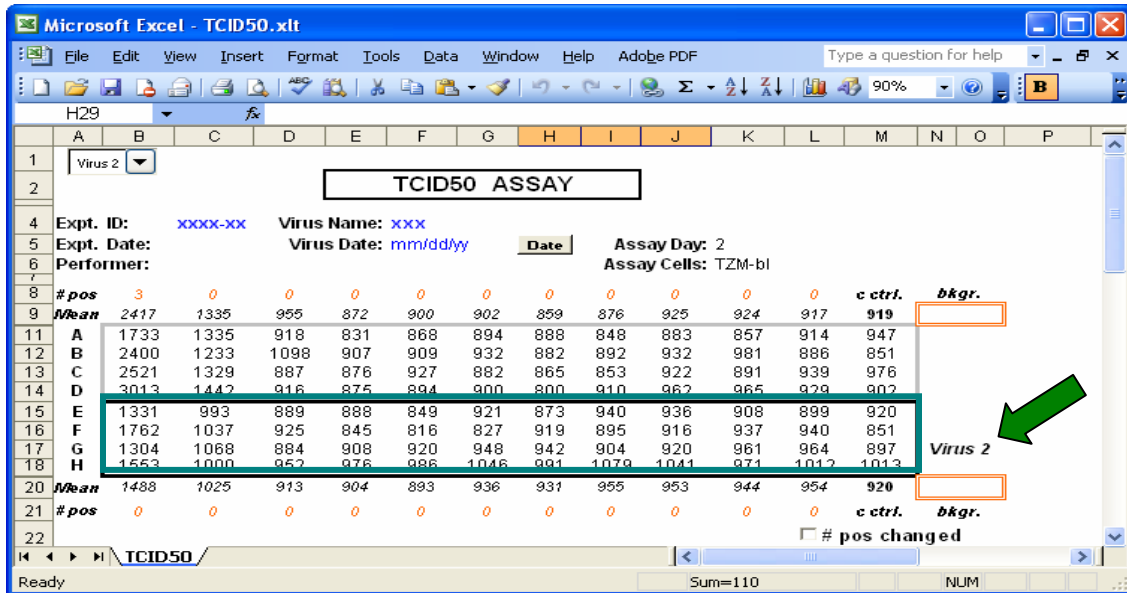
Select which virus to analyze.

TCID50 ASSAY													
4	Expt. ID:	xxxx-xx	Virus Name:	xxx									
5	Expt. Date:		Virus Date:	mm/dd/yy	Date	Assay Day:	2						
6	Performer:										Assay Cells:	TZM-bl	
8	# pos	3	0	0	0	0	0	0	0	0	c ctrl.	bkgr.	
9	Mean	2417	1335	955	872	900	902	859	876	925	924	917	919
11	A	1733	1335	918	831	868	894	888	848	883	857	914	947
12	B	2400	1233	1098	907	909	932	882	892	932	981	886	851
13	C	2521	1329	887	876	927	882	865	853	922	891	939	976
14	D	3013	1442	916	875	894	900	800	910	962	965	929	902
15	E	1331	993	889	888	849	921	873	940	936	908	899	920
16	F	1762	1037	925	845	816	827	919	895	916	937	940	851
17	G	1304	1068	884	908	920	948	942	904	920	961	964	897
18	H	1553	1000	952	976	986	1046	991	1079	1041	971	1012	1013
20	Mean	1488	1025	913	904	893	936	931	955	953	944	954	920
21	# pos	0	0	0	0	0	0	0	0	0	0	0	920
													bkgr.

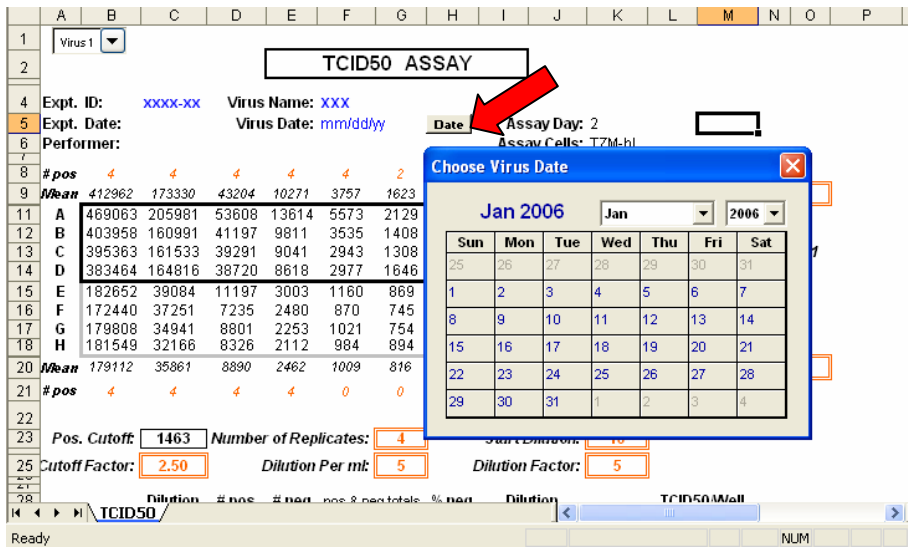
TCID50

pos changed

It will be the same procedure for Virus 2. If the Virus 2 is selected, the bottom half of the data box will be highlighted and Virus 2 will be shown next to the box.



7. Click **Date** button to select virus date.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	F
1	Virus 1															
2	TCID50 ASSAY															
4	Expt. ID:	xxxx-xx		Virus Name:	XXX			Date	Assay Day: 2							
5	Expt. Date:			Virus Date:	mm/dd/yy			Date	Assay Cells: T2M-bl							
6	Performer:															
7																
8	# pos	4	4	4	4	4	2	2	0	0			c ctrl.	bkg.		
9	Mean	412962	173330	43204	10271	3757	1623	1382	1060	972	838	795	585			
11	A	469063	205981	53608	13614	5573	2129	1790	1343	861	831	744	587	Virus 1		
12	B	403958	160991	41197	9811	3535	1408	1562	1238	1026	843	669	570			
13	C	395363	161533	39291	9041	2943	1308	1060	762	1133	618	896	565			
14	D	383464	164816	38720	8618	2977	1646	1115	898	867	1058	870	619			

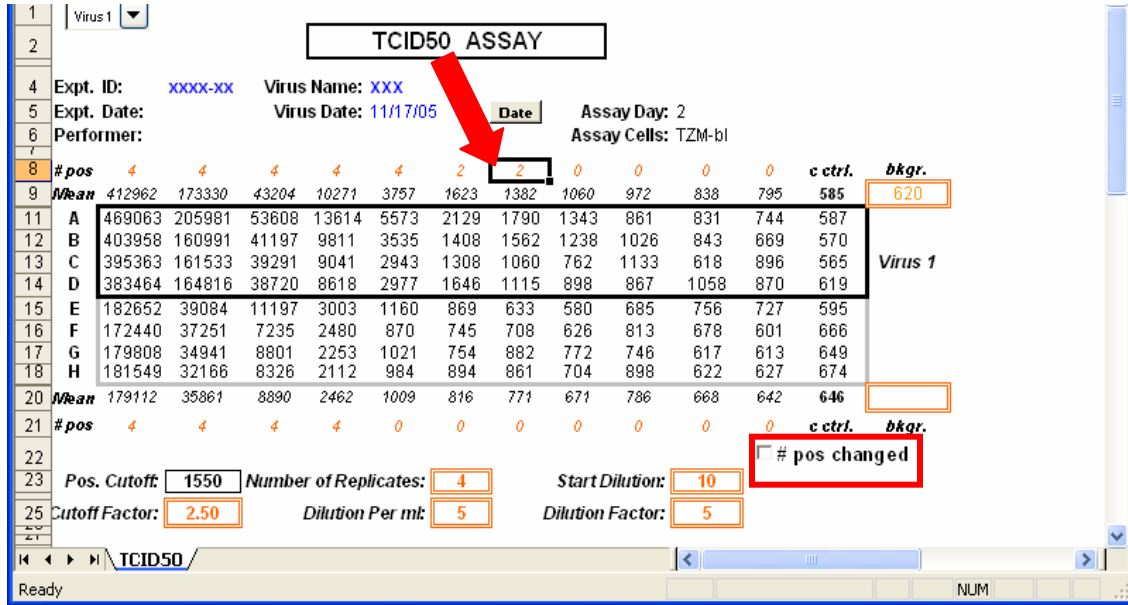
Drop down box to select assay cells.

8. Cells where the user may need to input different information, such as the cutoff factor, start dilution, dilution factor, etc are indicated by the double line orange box. Please enter the values directly into these double line orange boxes.

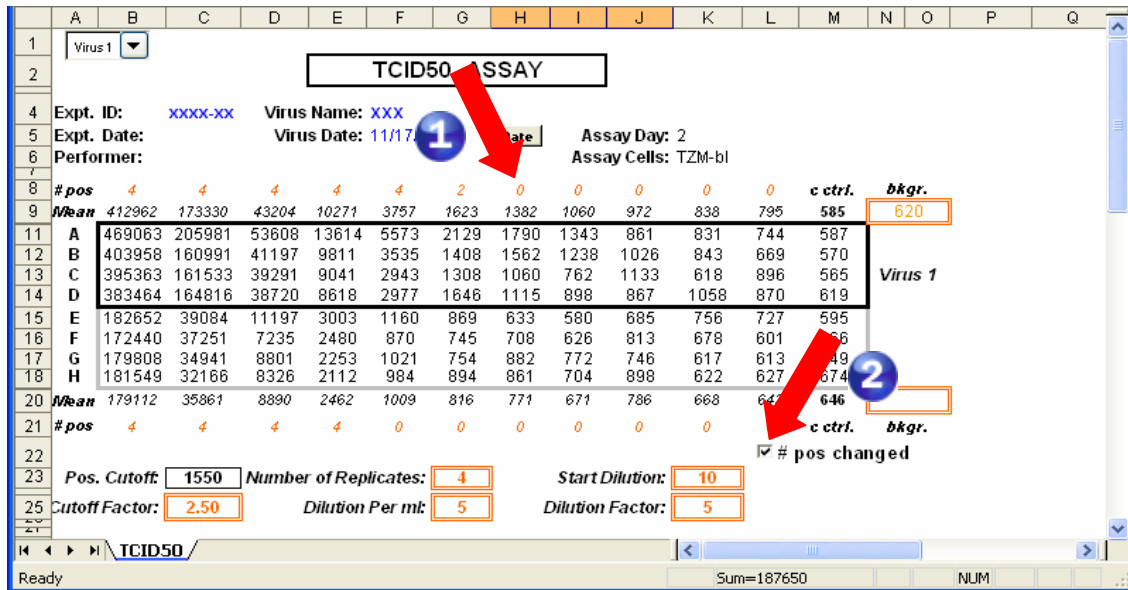
9. For the background value, leave it blank to use the default value which is next to the double line box. Or enter the value that you want to use into the double line orange box. For example, see figure below. The background value for Virus 1 is 620. If no value is in the double line box, it will use the value next to it—585.

8	# pos	4	4	4	4	4	2	2	0	0	0	0	c ctrl.	bkg.	
9	Mean	412962	173330	43204	10271	3757	1623	1382	1060	972	838	795	585	620	
11	A	469063	205981	53608	13614	5573	2129	1790	1343	861	831	744	587	Virus 1	
12	B	403958	160991	41197	9811	3535	1408	1562	1238	1026	843	669	570		
13	C	395363	161533	39291	9041	2943	1308	1060	762	1133	618	896	565		
14	D	383464	164816	38720	8618	2977	1646	1115	898	867	1058	870	619		
15	E	182652	39084	11197	3003	1160	869	633	580	685	756	727	595		
16	F	172440	37251	7235	2480	870	745	708	626	813	678	601	666		
17	G	179808	34941	8801	2253	1021	754	882	772	746	617	613	649		
18	H	181549	32166	8326	2112	984	894	861	704	898	622	627	674		
20	Mean	179112	35861	8890	2462	1009	816	771	671	786	668	642	646		
21	# pos	4	4	4	4	0	0	0	0	0	0	0	c ctrl.	bkg.	

10. The user may wish to change the number of wells positive for infection. First change the #pos directly and then put a check mark in the check box # pos changed (indicated by red box in the figure below). For example, below we need to change the number of positive from 2 to 0 (indicated by the red arrow below).



(Before)



(After)

The Figure above shows the 2 steps to change the number of positive.

Step 1: Change 2 to 0.

Step 2: Put a check mark in the check box.

For questions or comments regarding the macro, please contact Peter Gao (juanfei.gao@duke.edu), Duke Central Immunology Laboratory.