



Excel Template Use Instructions

Welcome to the Muta-ChromoPlate Excel Template User Instructions. Following the simple steps outlined below, you will be able to quickly enter your experimental results and have it analyzed in seconds, providing a quick and easy way to obtain results.

To begin entering data, open the Muta-ChromoPlate Excel Template file under the Muta-ChromoPlate folder on the CD-ROM included in your kit. Also included in this folder is a file entitled “Muta-ChromoPlate Complete Example”, which serves as an additional reference for proper data entry.

Step 1: Enable Macros

In order to be able to have Excel automatically be able to analyze your results, it will have to run a macro written in VBA code (see Step x on how to run the macro).

This is a very simple procedure to perform. The procedure is provided for both Excel 2003 and Excel 2007.

Excel 2003¹:

When the macro security level in Excel is set to **Low** (not recommended), macros can be run without prompting. When macro security is set to **Medium**, Excel displays a dialog box asking if you want to enable macros. When macro security is set to **High** (the recommended macro security setting for all users), Excel allows you to run only those macros that are digitally signed or stored in the Excel startup (XLStart) folder.

¹ See Support/Excel/Excel 2003 Help and How-to/Security and Privacy/Macros and Virus Protection at <http://office.microsoft.com/en-us/excel-help/about-macro-security-HP003084611.aspx?CTT=5&origin=HP010096919> , Microsoft, 2011

Enable an unsigned macro to run

To allow unsigned macros to run, the **Trust all installed add-ins and templates** check box must be selected on the **Trusted Publishers** tab of the **Security** dialog box. This option is selected by default. If it is not selected (recommended), Excel allows you to run only macros that have trusted digital signatures.


1. On the **Tools** menu, point to **Macro**, and then click **Security**.
2. On the **Trusted Publishers** tab, select the **Trust all installed add-ins and templates** check box

Excel 2007:

Microsoft Office Excel allows you to change the macro security settings in order to control which macros run and under what circumstances. The first thing to check is that macros are not entirely disabled, as this will prevent the use of the analytical macro.


You can change macro security settings in the Trust Center, unless a system administrator in your organization has changed the default settings to prevent you from changing the settings.

1. On the **Developer** tab, in the **Code** group, click **Macro Security**.

Tip If the **Developer** tab is not displayed, click the **Microsoft Office Button** , click **Excel Options**, and then in the **Popular** category, under **Top options for working with Excel**, click **Show Developer tab in the Ribbon**.

2. In the **Macro Settings** category, under **Macro Settings**, click the option that you want.

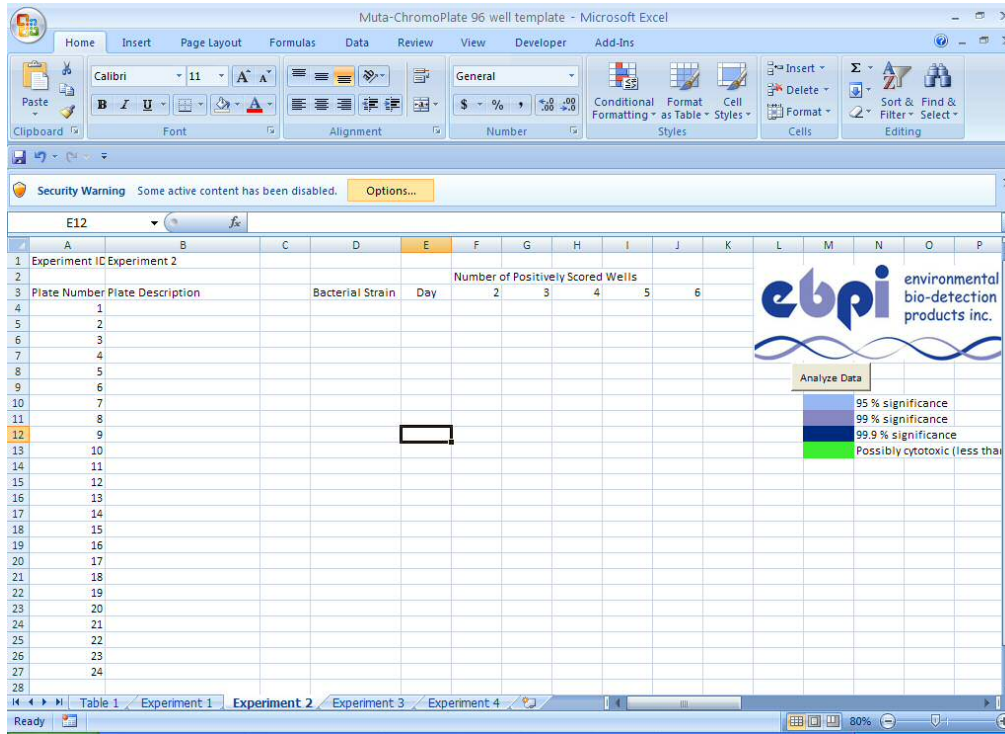
Note Any changes that you make in the **Macro Settings** category in Excel apply only to Excel and do not affect any other Microsoft Office program.

Tip You can also access the Trust Center in the **Excel Options** dialog box. Click the **Microsoft Office Button** , and then click **Excel Options**. In the **Trust Center** category, click **Trust Center Settings**, and then click the **Macro Settings** category.

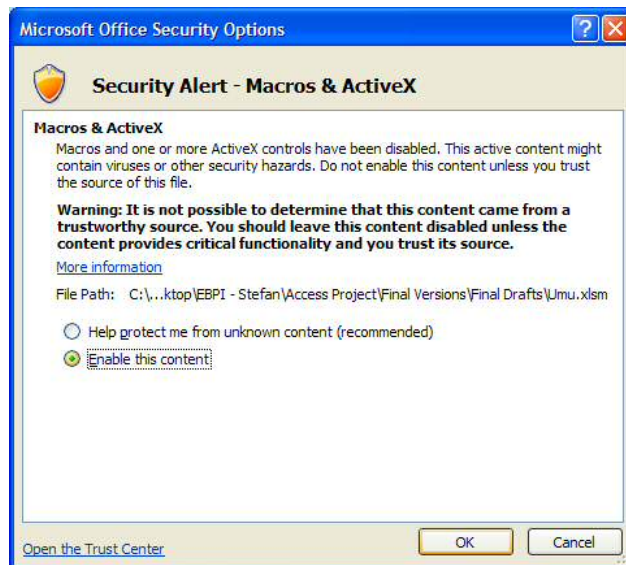
The default macro setting for Excel is “Disable all macros with notification”. This means that Excel will prompt you when spreadsheets have macros, and you can choose whether to trust the source and use the macro, or to ignore it. In this case, you will want to enable macros in order to facilitate data analysis.²

² See Support/Excel/Excel 2007 Help and How-to/Macros at <http://office.microsoft.com/en-us/excel-help/change-macro-security-settings-in-excel-HP010096919.aspx>, Microsoft, 2011

If you changed the macro security settings, you will want to restart Excel in order for the changes to occur. Once the macro security setting is set to “Disable all macros with notification”, a Security warning will appear below the task bar (see picture below).



Simply click on “Options...” at which point the following window will appear:

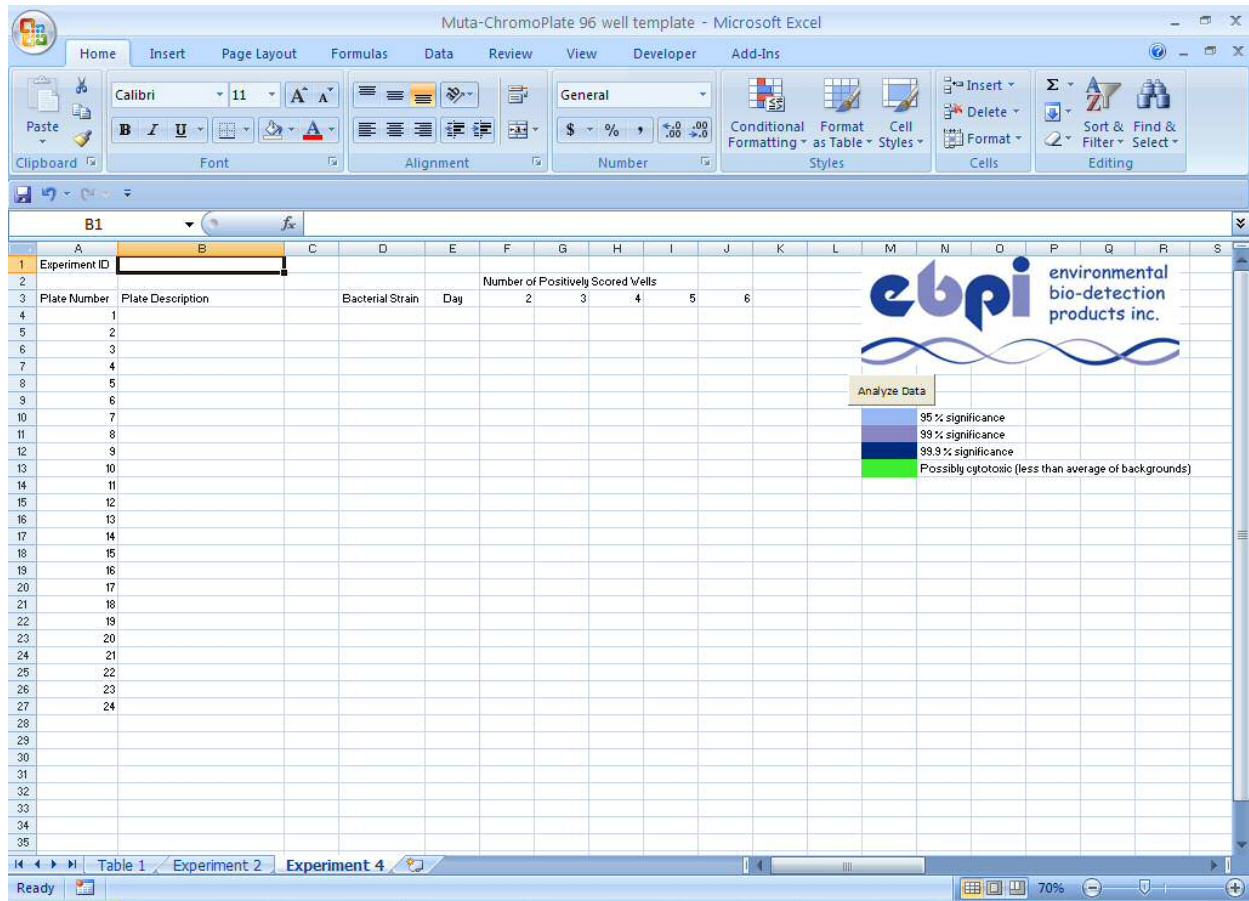


Choose “Enable this content” and click OK.

You are now ready to enter data and perform data analysis. It is important to protect your computer and data files by not allowing every macro to run automatically. For this reason, the default security setting for macros is suggested. Simply follow these steps for any EBPI product with accompanying software in order to protect yourself.

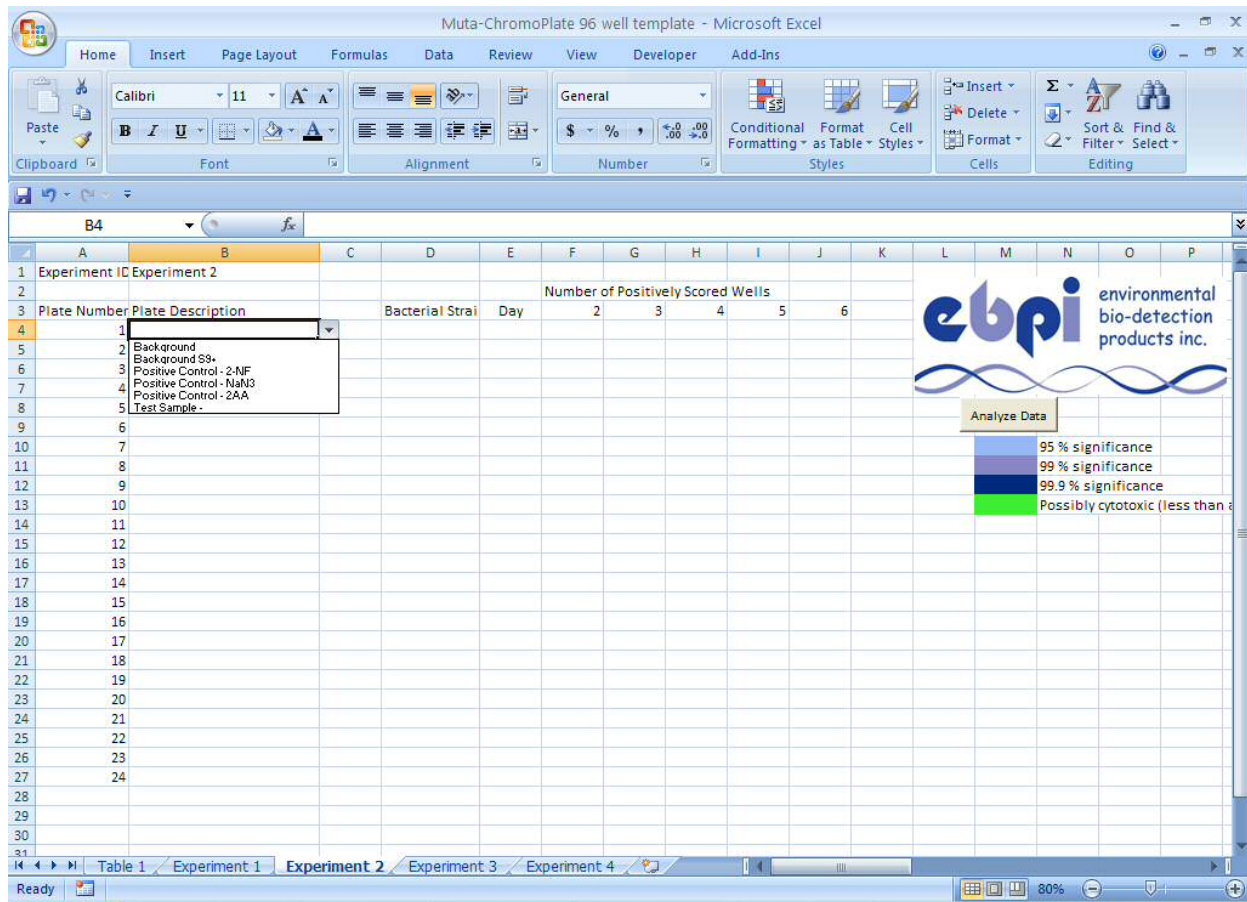
Step 2: Data Entry and Analysis

Below is an image of the template as it appears when you first open the Muta-Chromoplate 96 well template Excel file.



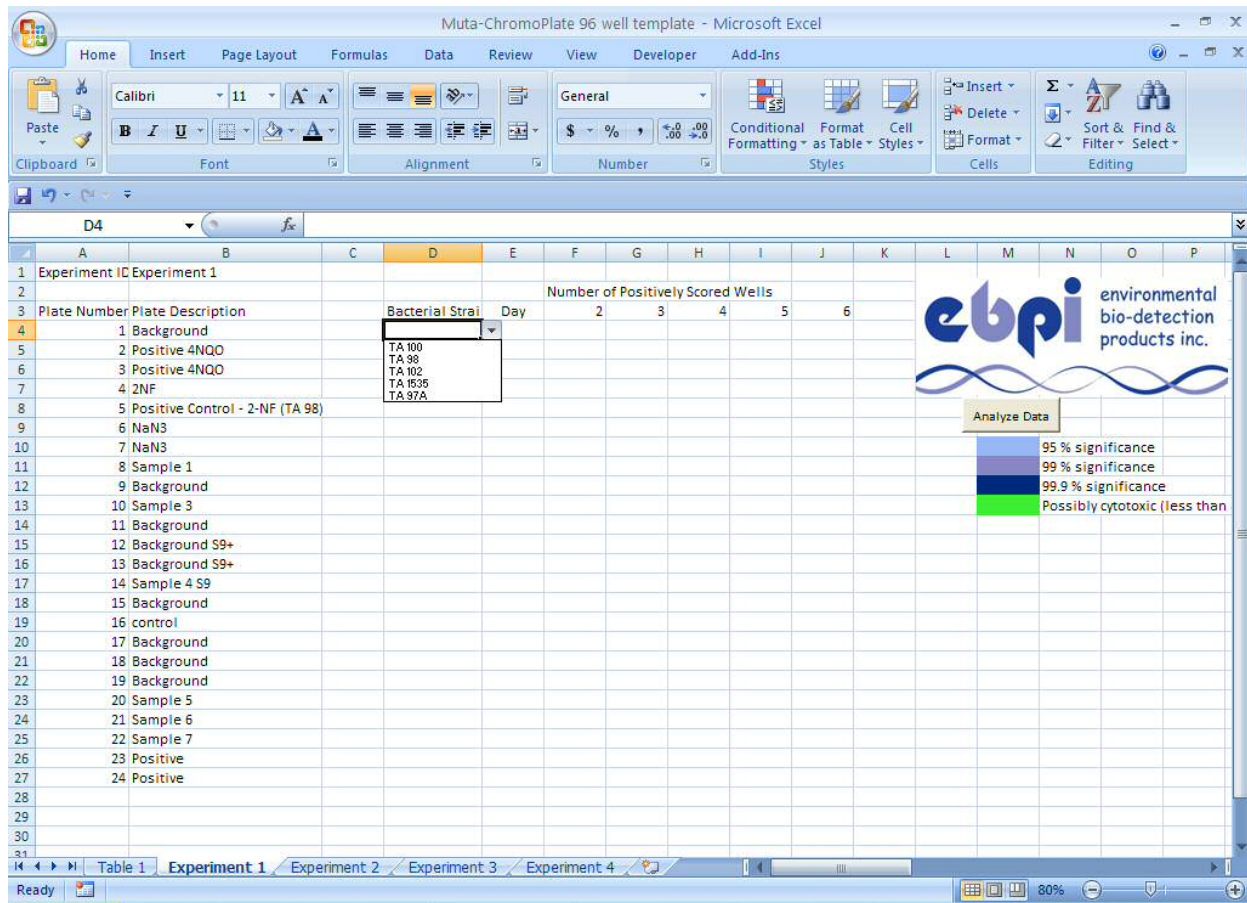
This template allows for:

- entry of as many plates as you would like onto a single sheet. If you wish to include more than 24 plates on a single spreadsheet (according to experimental design), simply increase the plate numbers accordingly.
- Entry of a unique Experimental ID in cell B2 (highlighted). This will rename the sheet according to the ID provided. It is important to have a unique name as Excel will not allow two sheets to have the same name.
- Entry of up to 3 background plates for each strain in a single experiment



To begin data entry, you will need to provide a Plate Description in Column B for each and every plate used. It is important to provide a plate description, as this is what is used by the macro in order to categorize the different plates for analysis. To this end, a drop-down menu (shown above) is provided in order to streamline this process. There are a number of preset selections, such as Background, Background S9+, Positive Controls and Test Sample. Although you may type any text into this field, it is necessary to use the selection for background samples and positive controls. Test Samples may be give any name, however, they cannot include the text “Positive”, “Control”, “NaN3”, “2-NF” or “Background” for obvious reasons.

It is also very important to note that in order to indicate whether a plate contains the S9 rat liver extract, the text “S9” must appear somewhere in the Plate Description.



After all of the plate descriptions have been entered, the next step is to enter the bacterial strain used in the experiment. It is not necessary to enter the bacterial strain for all plates. Simply enter the strain once, and all subsequent plates will be designated as that strain. If your experimental design included the use of more than one strain, then simply enter a new strain name for the appropriate plates (see next page for example of experimental design with several strains used).

For this Column, a drop-down menu has been provided with 5 of the different strains available from EBPI. It is not possible to enter other strains, as there are a number of different tolerances used in the macro according to the strains chosen, with only EBPI strains supported.

Muta-ChromoPlate 96 well template - Microsoft Excel

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Clipboard Font Alignment Number Styles Cells Editing

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1	Experiment ID	Experiment 2			Number of Positively Scored Wells					
3	Plate Number	Plate Description	Bacterial Strain	Day	2	3	4	5	6	
4	1	Background	TA 100		6	7	8	9	13	
5	2	Positive 4NQO			75	75	75	75	75	
6	3	Positive 4NQO			75	75	75	75	75	
7	4	2NF			57	74	74	74	74	
8	5	Positive Control - 2-NF (TA 98)			75	75	75	75	75	
9	6	NaN3			75	75	75	75	75	
10	7	NaN3			75	75	75	75	75	
11	8	Sample 1			20	22	24	26	26	
12	9	Background			17	21	24	26	26	
13	10	Sample 3	TA 98		20	21	24	26	26	
14	11	Background	TA 100		8	9	10	11	12	
15	12	Background S9+			12	13	14	15	16	
16	13	Background S9+			13	14	15	16	17	
17	14	Sample 4 S9			12	22	24	26	26	
18	15	Background	TA 98		2	3	4	5	6	
19	16	control	TA 100		81	82	83	95	95	
20	17	Background	TA 98		4	5	6	7	8	
21	18	Background	TA 102		6	7	8	9	10	
22	19	Background	TA 1535		5	6	7	8	9	
23	20	Sample 5	TA 102		15	19	22	25	32	
24	21	Sample 6	TA 1535		12	24	27	30	37	
25	22	Sample 7			15	29	32	35	42	
26	23	Positive			75	75	75	75	75	
27	24	Positive	TA 102		85	85	85	85	85	

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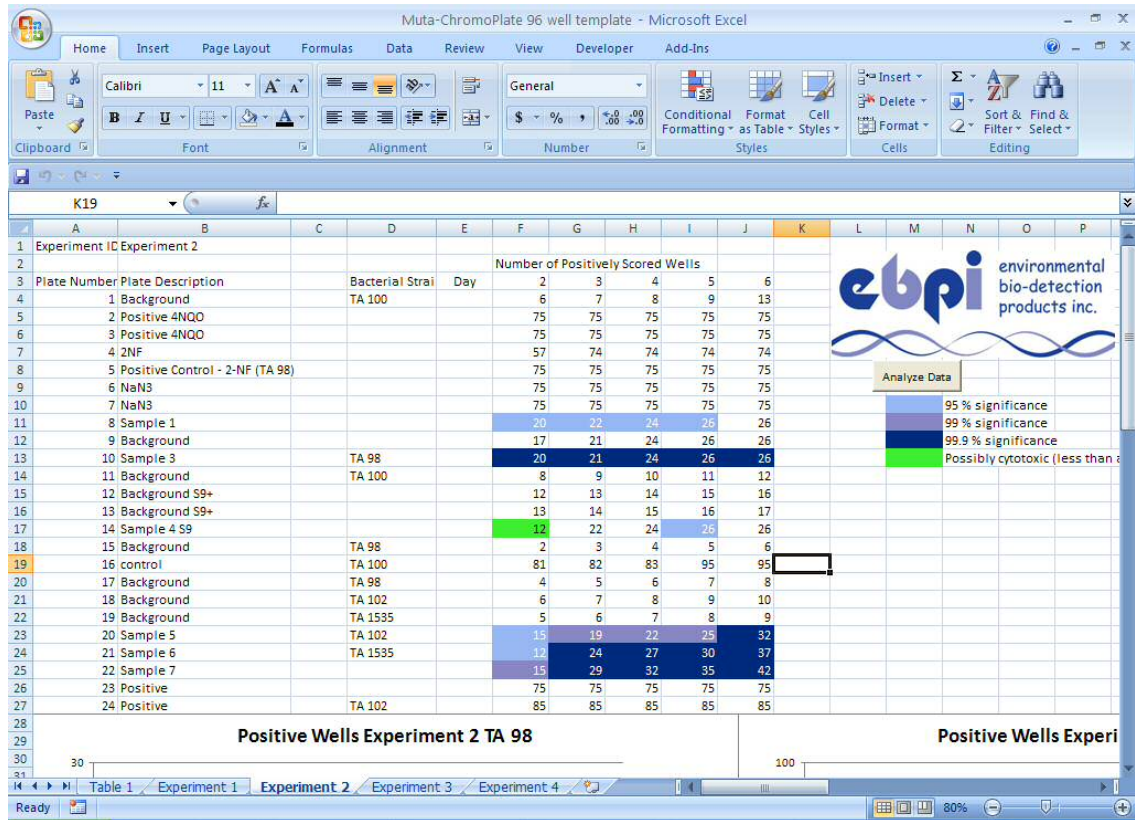
Analyze Data

- 95 % significance
- 99 % significance
- 99.9 % significance
- Possibly cytotoxic (less than 85)

Table 1 Experiment 1 Experiment 2 Experiment 3 Experiment 4

Select destination and press ENTER or choose Paste 80%

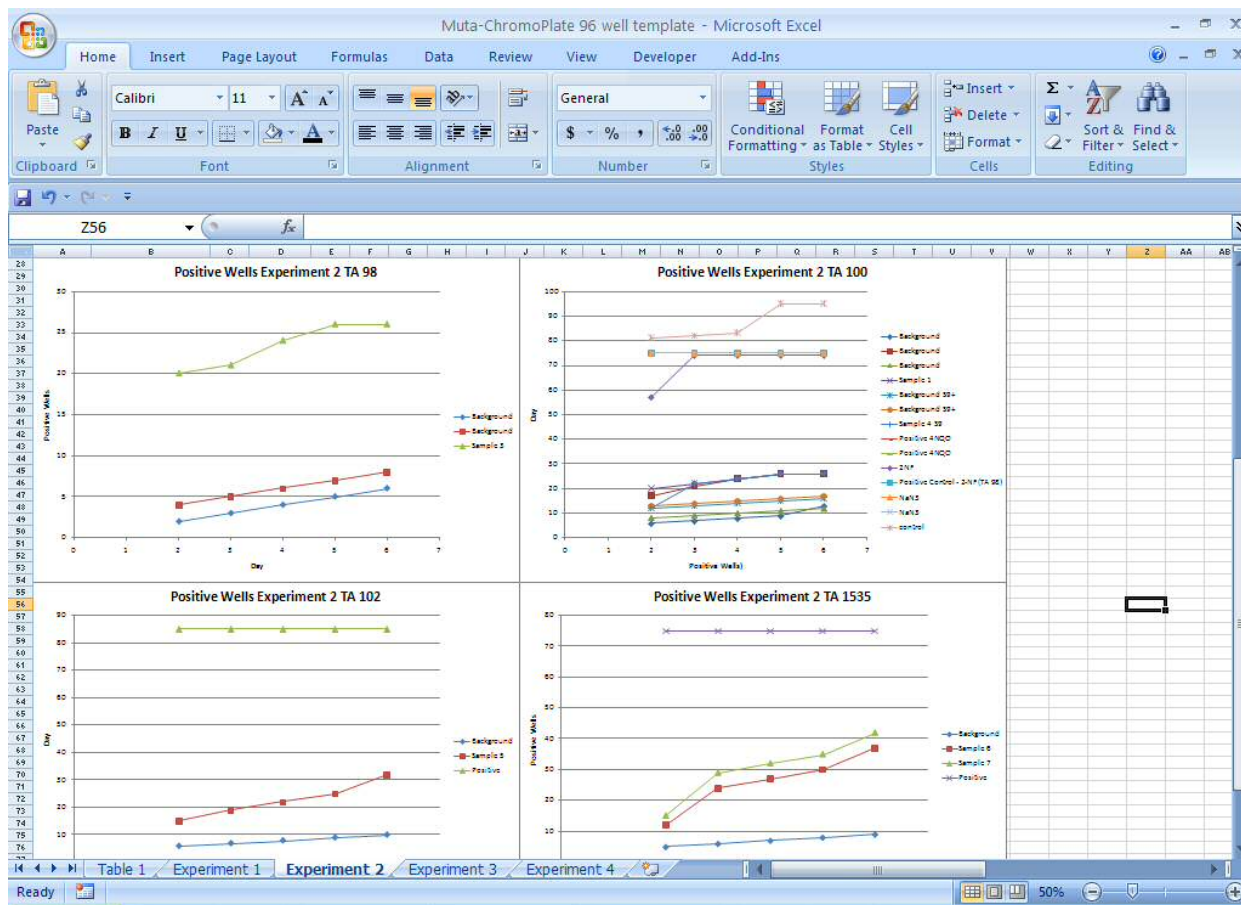
Once the bacterial strains have been provided, you can enter the number of positively scored wells for each plate for the duration of your experiment.



After data for all the plates has been entered, simply click on the “Analyze Data” button found just beneath the EBPI logo. Now Excel will run a macro which colour-codes cells according to the legend found beneath the “Analyze Data” button as well as generating graphs for each strain showing the increase in positive wells for the span of the experiment, allowing for visual comparison of samples, backgrounds and controls.

As a reminder, all yellow, partially yellow or turbid well are scored as positives and all purple wells are scored negative (compare to negative control plates). For each treatment, the statistical significance of the difference between the sample and the average of the background plates are computed using Table 3 in the Instruction Manual accompanying your Muta-ChromoPlate kit. The higher the significance, the more likely that the additional positive wells are explained by the presence of the test sample, and not random mutation.

As shown in the legend, light blue shows that there is only a 5% chance that the difference in positively scored wells between the Sample plate and average of the backgrounds for that strain are explained by spontaneous mutation (with light purple showing a 1% chance and dark blue showing 0.1% chance). Finally, a cell is shaded green if it has less positively scored wells than the average of the backgrounds for that strain. This could indicate cytotoxicity since it has less mutated cells than the control cells which represent the natural, spontaneous mutation rate.



As well as colour-coding the data in order to denote significance of the results, the macro will also generate graphs for each strain. Pictured above are the results for the four strains in the example. All plates for a strain are included in that graph, allowing for easy comparison of the samples, backgrounds and controls. You are also free to create your own custom graphs if you would like to look at other comparisons more directly.

As a final note, it is recommended that you delete all the charts if you choose to run the macro a second time, as the new charts will simply cover up the old charts, which can eventually lead to a larger draw on system memory, slowing Excel and your computer.

If you have any technical issues with the spreadsheet or further inquiries, please do not hesitate to contact EBPI at sales@biotoxocity.com or by phone at 905-487-7359.