



## Excel Template Use Instructions

Welcome to Umu-ChromoTest 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 Umu-ChromoTest Excel Template file located in the Umu-ChromoTest folder on the CD-ROM included in your kit. Also included in this folder is a file entitled “Umu-ChromoTest 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 2003<sup>1</sup>:**

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.

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<sup>1</sup> 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.

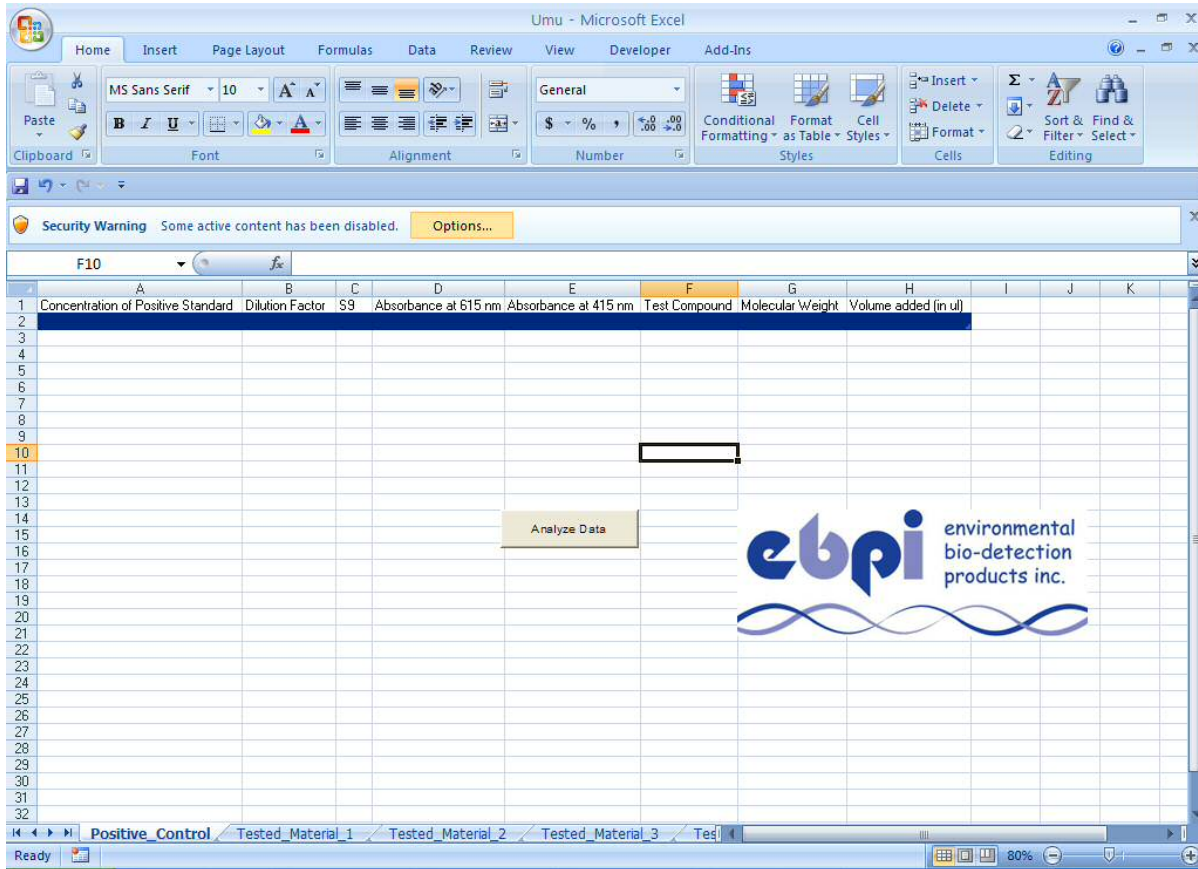
**Tip** You can also access the Trust Center in the **Excel Options** dialog box. Click the **Microsoft Office Button** , and then click **Excel Options**. In the **Trust Center** category, click **Trust Center Settings**, and then click the **Macro Settings** category.

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.<sup>2</sup>

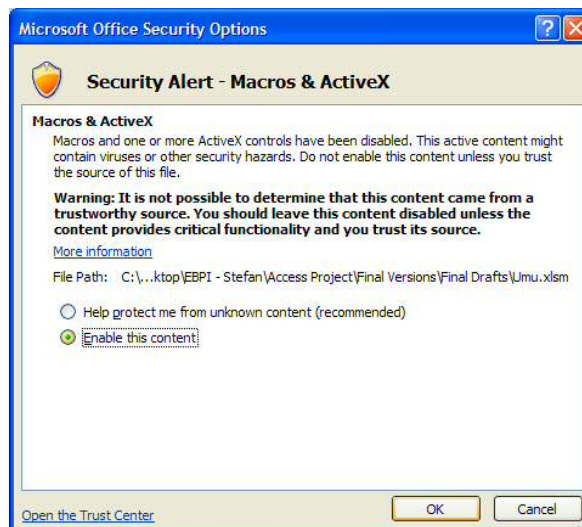
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<sup>2</sup> See Support/Excel/Excel 2007 Help and How-to/Macros at <http://office.microsoft.com/en-us/excel-help/change-macro-security-settings-in-excel-HP010096919.aspx>, Microsoft, 2011

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:

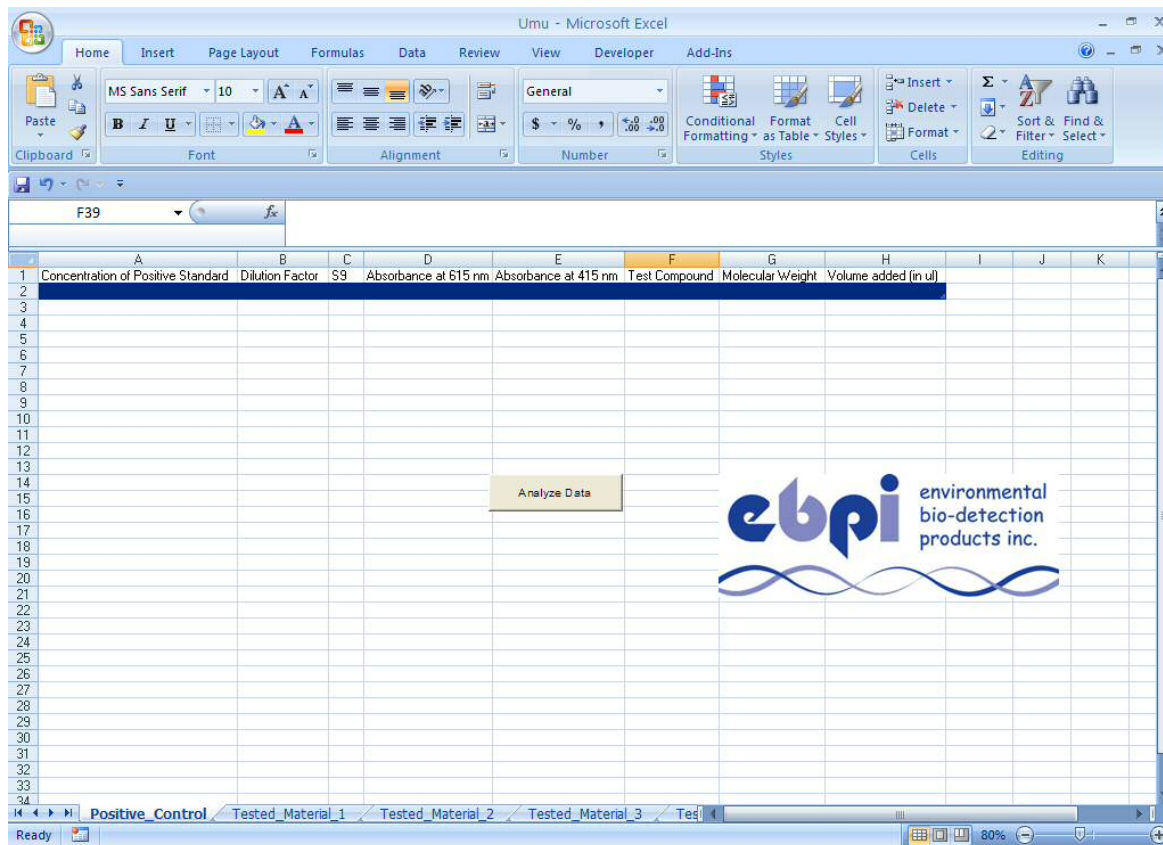


Choose “Enable this content” and click OK.

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

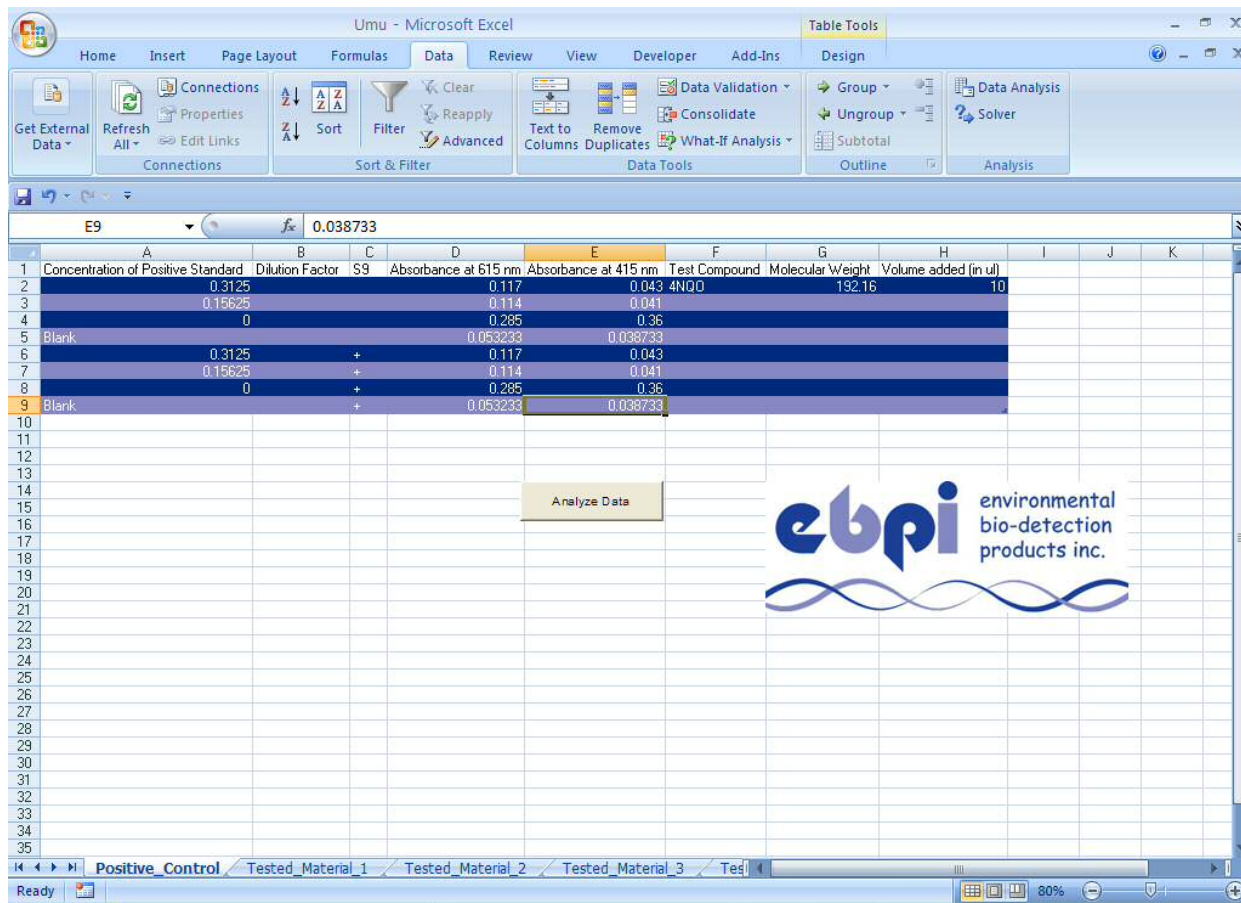
Below is an image of the template as it appears when you first open the Umu-ChromoTest Excel Template file.



This template allows for entry of up to 6 samples per plate, as well as a positive control. From the image above, you can see that the positive control and each sample are separated onto different worksheets within the Excel file in order to better organize the data.

As the first step in data entry, it is recommended to first fill out the Positive Control worksheet. Simply enter the concentration of the positive control material in Column A, or enter the dilution factor into Column B. It is important to pick either one or the other. It is also important to note that if you are using dilution factors for either the positive control or any of the test samples, you must indicate the negative control well by typing “Negative” into the appropriate cell. Finally, enter the absorbance at 600nm (+/- 20 nm) in Column D and the absorbance at 420 nm (+/- 20nm) in Column E for each concentration.

Also you may enter information about the positive control, as well as all the test samples in Columns F-H where you may enter a description, molecular weight and the volume added in ul, respectively. If you do not provide the molecular weight or the volume added for any of the test samples, then the analysis will be incomplete.



One important thing to note is that the blank wells should be included in the Positive Control worksheet. In order to indicate the presence of a blank, simply type “Blank” into the concentration of dilution factor column (depending on which you are using).

As well as providing a comparison between samples, this file allows for comparison between samples with S9 rat liver extract and samples without. To indicate whether a sample has S9 rat liver extract included, you may use the drop down menu in Column C in order to place a “+” sign in the appropriate cell. You may enter other values or characters in place of the “+” sign, however, it is essential to have at least one character in Column C for the appropriate row to designate it as being metabolically activated for the macro to run properly.

1	A	B	C	D	E	F	G	H	I
2	Concentration of Test Material 1	Dilution Factor	Absorbance 615 nm	Absorbance 415 nm	Test Compound	Molecular Weight	Volume added (in ul)		
3		1.5	2.06663	0.308	Sample 1				
4		3	1.3323	0.323366667					
5		6	0.7677	0.3134					
6		12	0.4821	0.319333333					
7		Negative	0.285	0.360033333					
8		1.5 +	2.06663	0.308					
9		3 +	1.3323	0.323366667					
10		6 +	0.7677	0.3134					
11		12 +	0.4821	0.319333333					
12		Negative +	0.285	0.360033333					

Data for test samples is entered identically to the positive control. Simply enter the concentrations or, if concentrations are unknown, the dilution factors used (with the text “Negative” to indicate the negative control). Enter the absorbance for each concentration at both 600 ( $\pm 20$ ) nm and 420 ( $\pm 20$ ) nm and any known details of the test compound.

You may enter either dilution factors or concentrations for the test samples, regardless of which was used for the positive control, since only negative controls and blanks are used in calculations for each test sample.

Once you have entered data for all test samples used, you are ready to run the macro in order to analyze the data.

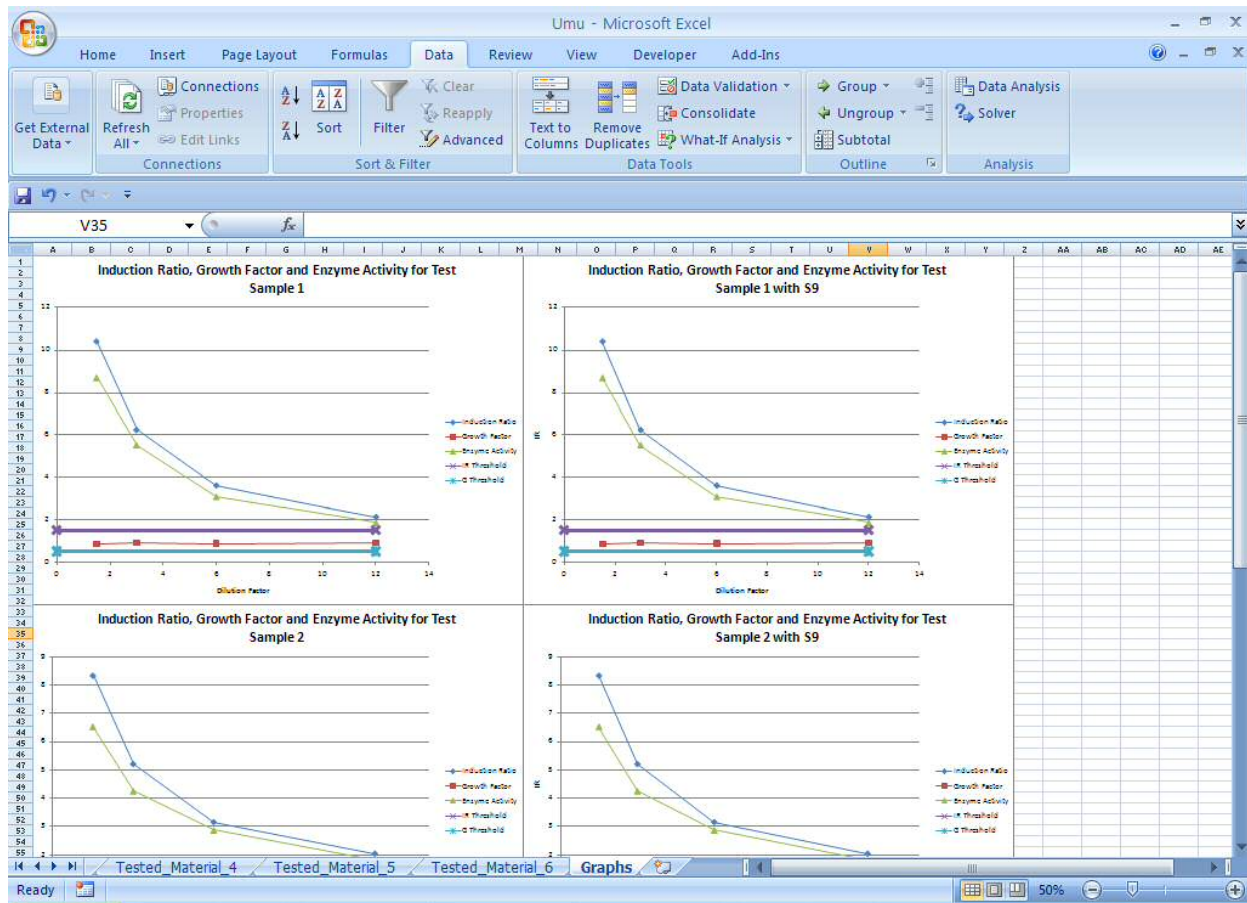
	K	L	M	N
	IR values	Growth Ratio	Enzyme Activity	Lowest Ineffective Dilution
2	3.1147325	0.83845	8.68716	>12
3	2.2847016	0.88597	5.51876	
4	1.15-151.24	0.85495	3.0827	
5	1.1163239	0.87342	1.85042	
6				>12
7	3.1147325	0.83845	8.68716	
8	2.2847016	0.88597	5.51876	
9	1.15-151.24	0.85495	3.0827	
10	1.1163239	0.87342	1.85042	

Once the data is entered for all test compounds used (you may leave tabs unused if you used less than 6 different samples), simply click on the “Analyze Data” button on the “Positive\_Control” worksheet.

Once “Analyze Data” is clicked, you will be asked to save a copy of the workbook in order to protect your data. A descriptive and unique name is suggested, including the date and purpose of experiment. Once you have saved or clicked on “Cancel”, the spreadsheet will analyze the data, giving numerical and graphical results.

You will have to scroll to the right in order to see the full analysis. Displayed in columns K-N are the Induction Ratio, Growth Ratio, Enzyme Activity and Highest Ineffective Concentration (or Lowest Ineffective Dilution) for each concentration (or dilution) of test sample. You will also notice that some of the values may be colour-coded. As described in the legend, a green cell indicates that the growth ratio is greater than 1, which implies the bacteria is at a higher concentration in the well containing the test sample at the concentration indicated by the row than the negative control, which can imply experimental error. Secondly, if the cell is

coloured red, it implies that the growth ratio is less than 0.5, indicating the test compound inhibited growth more than 50% compared to the negative control, and Induction Ratio values calculated may not be reliable. Finally, if the cell is coloured blue, it implies that the sample may be genotoxic at the current concentration as the Induction Ratio is greater than 1.5 and the growth ratio is not less than 0.5. The Highest Ineffective does indicates the highest concentration or dilution that is less than the 1.5 induction ratio threshold for genotoxicity. If all the induction ratio values are above 1.5, then this value will indicate such.



The macro will also generate a new worksheet entitled “Graphs” where it will display a graph for each sample entered, as well as an additional graph if S9 added to the sample in some wells, allowing for easy comparison of the genotoxicity of the sample with and without enzymatic activation.

Each chart plots the Induction Ratio, Enzyme Activity and Growth Ratio for the full range of concentrations or dilution factors. Also present are horizontal lines representing the threshold of Induction Ratios for genotoxicity (>1.5) as well as one for Growth Ratios to show the threshold for cytotoxicity (< 0.5).

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@biotoxycity.com](mailto:sales@biotoxycity.com) or by phone at 905-487-7359.