
Excel Template Use Instructions

Welcome to SOS-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.

**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**[1]:

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.

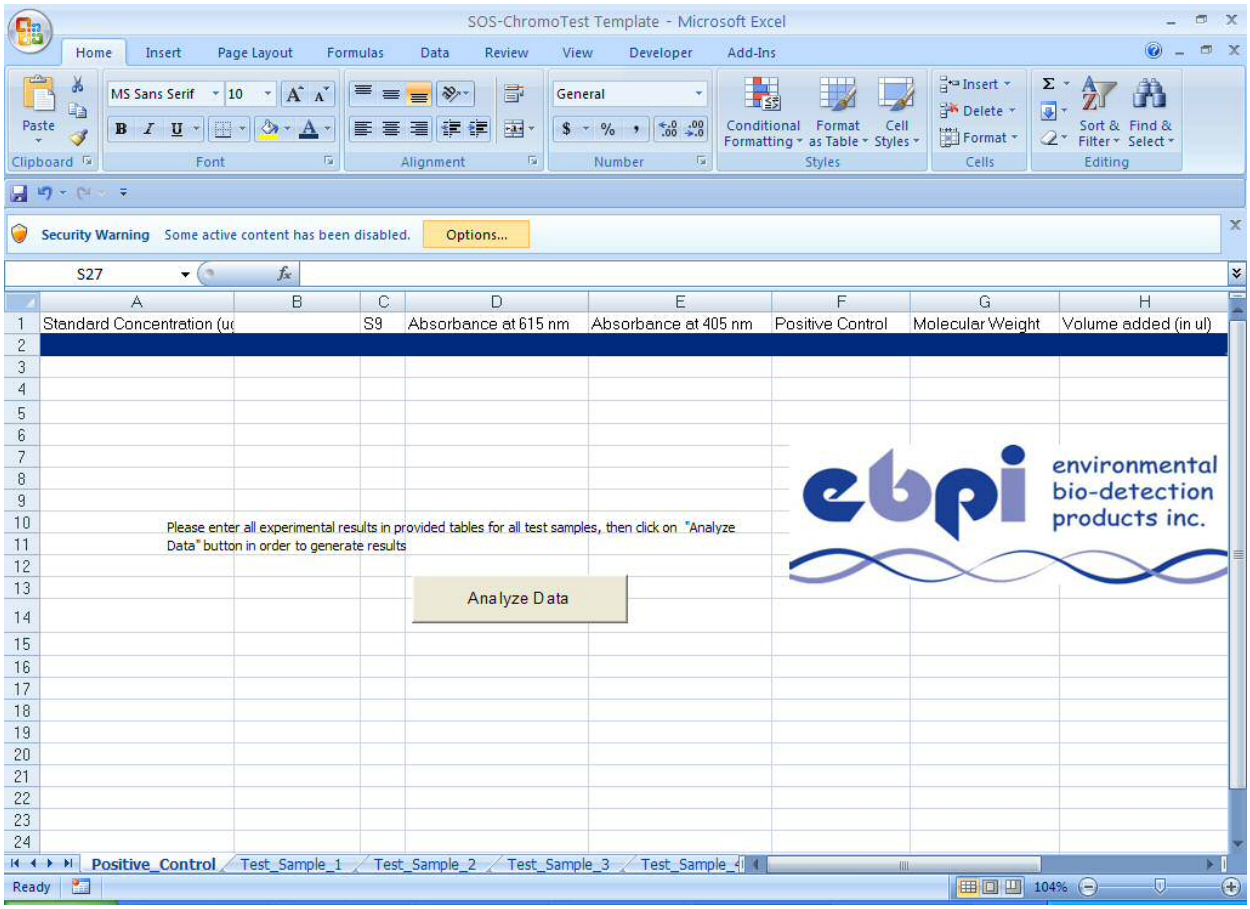
**Note** Any changes that you make in the **Macro Settings** category in Excel apply only to Excel and do not affect any other Microsoft Office program.

**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.

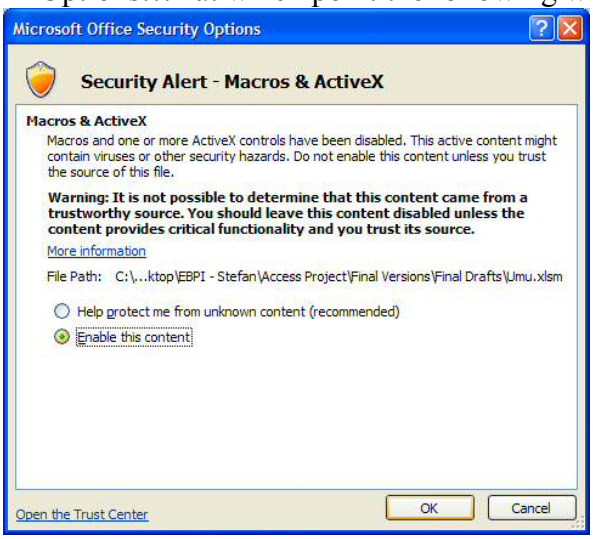
The default macro setting for Excel is "Disable all macros with notification". This means that Excel will prompt you when spreadsheets have macros, and you can choose whether to trust the source and use the macro, or to ignore it. In this case ,you will want to enable macros in order to facilitate data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

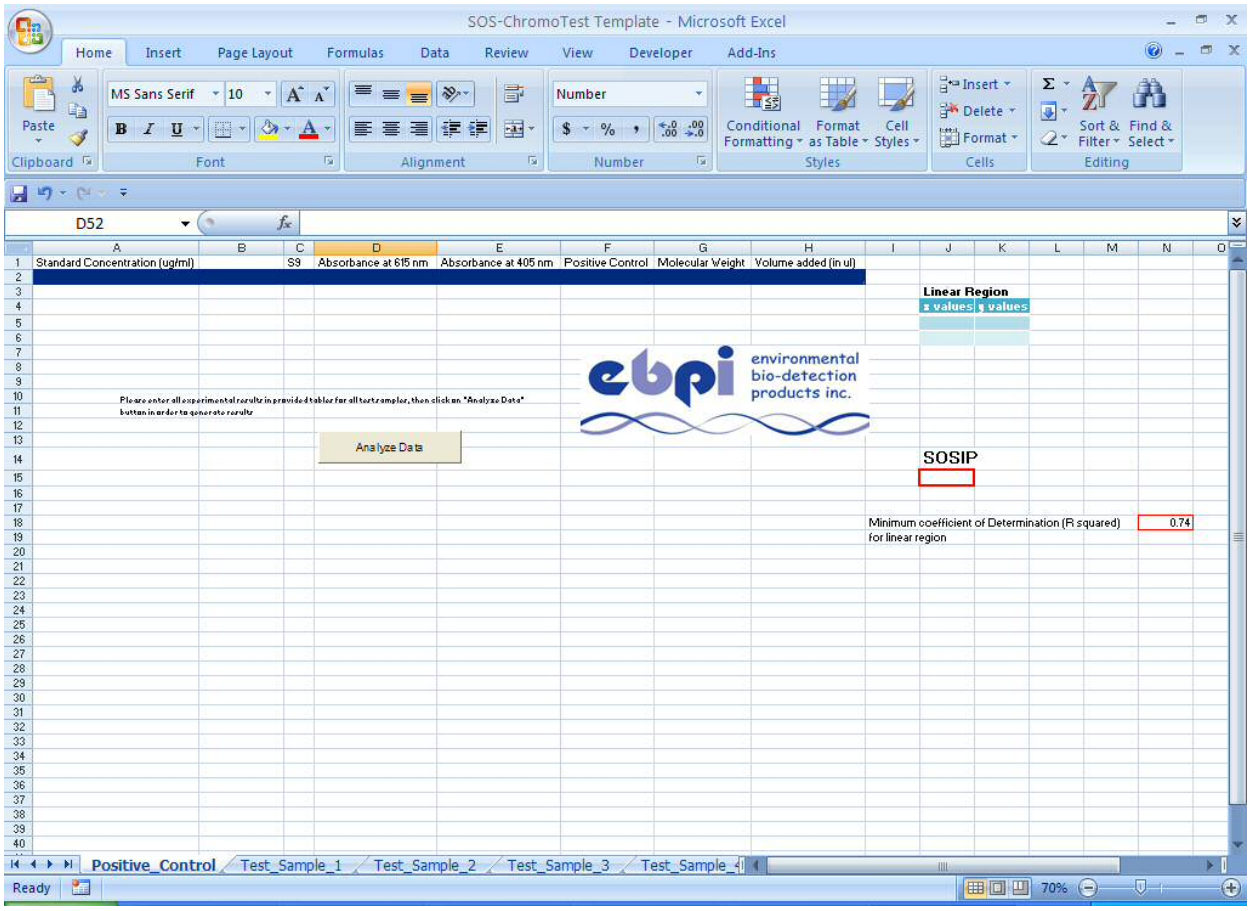



Choose "Enable this content" and click OK.

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 SOS-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.

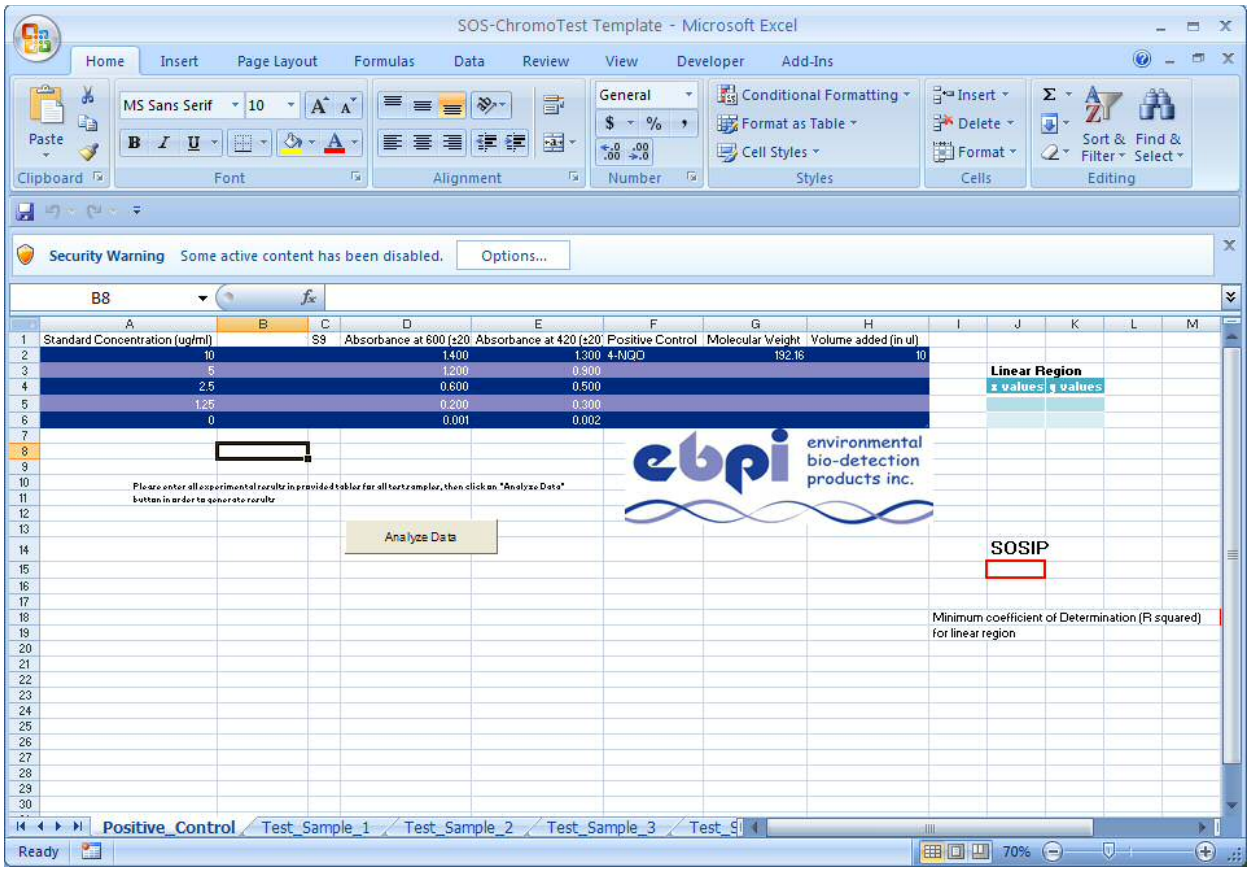
As a preliminary step before data entry, it is necessary to set some bounds for different factors related to the determination of the SOSIP. As you can see on the lower right of the picture above (in cells “N18” and “N20”), you required to provide two values. The first, the coefficient of determination, sets the minimum $R^2$value for the line of best fit through the linear regionof the plot of the absorbance vs concentration for each sample as shown in Figure 1 of the instruction manual ac companying the SOS-Chromotest. For this example,a value of 0.74 has been chosen, although the default is set

at 0.90. The value is included in order to make it easier for the user to explore results if the user interprets the data differently than the macro.

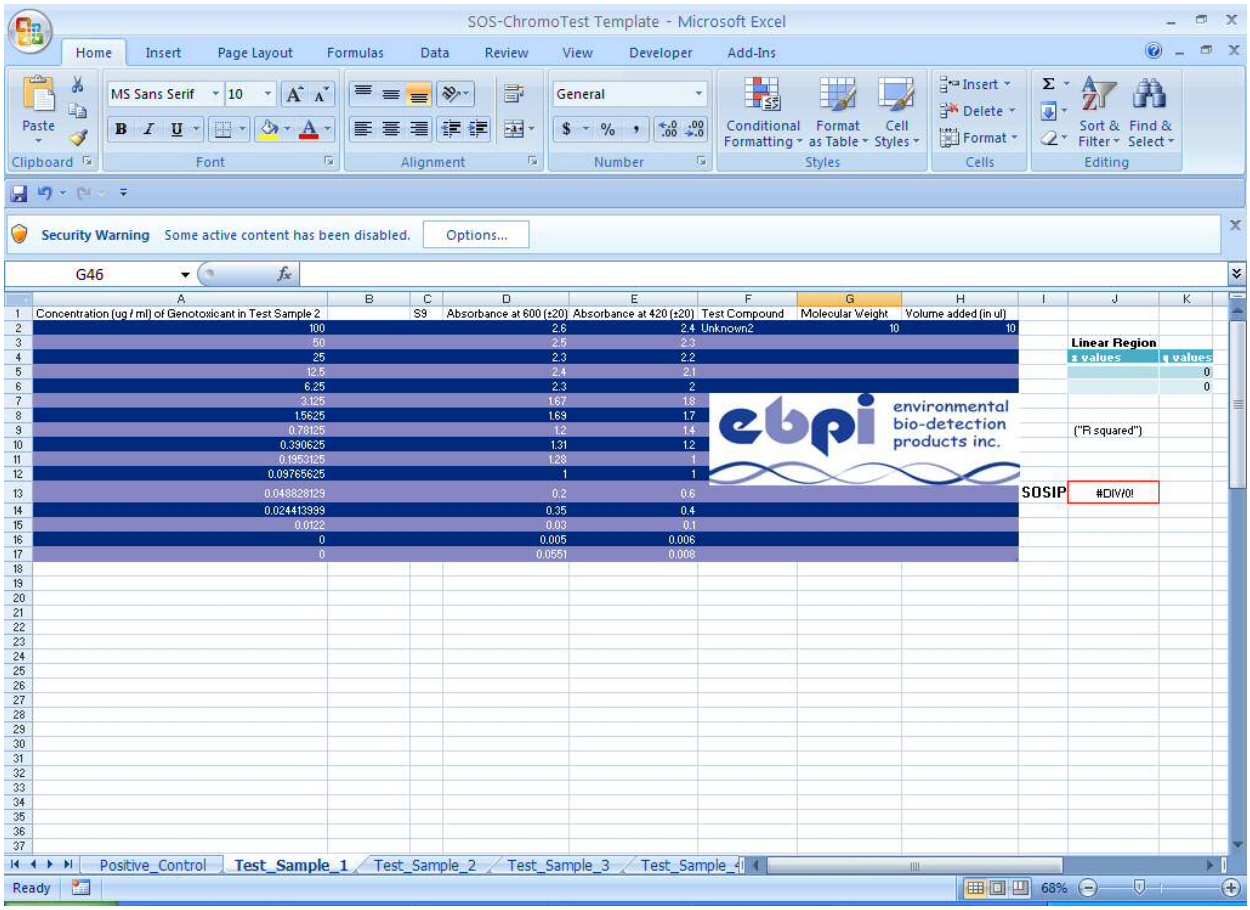
As the first step in data entry, it is recommended to fill out the Positve Control worksheet. Simply enter the concentrations of the positive control materia in Column A. Then enter the absorbencies at 600 (±20) nm and 420 (±20) nm fo each concentration.

Also it is necessary to enter the molecular weight of the positive control (the molecular weight of all controls are listed in the instruction manuals accompanying the SOS-ChromoTest kit), as well as the volume added. These values are required in order to calculate the SOS-Induction Potential (SOSIP) of the positive control, which is used in the calculation of the SOSIP's for all of the test samples.

Finally, it is important to note that you should use a separate file for samples which contain the S9 fraction and for those samples without S9. This is due to the fact that the samples need to be compared to their respective positive controls, and using two sheets allows for data entry sheets to be as straighforward as possible.



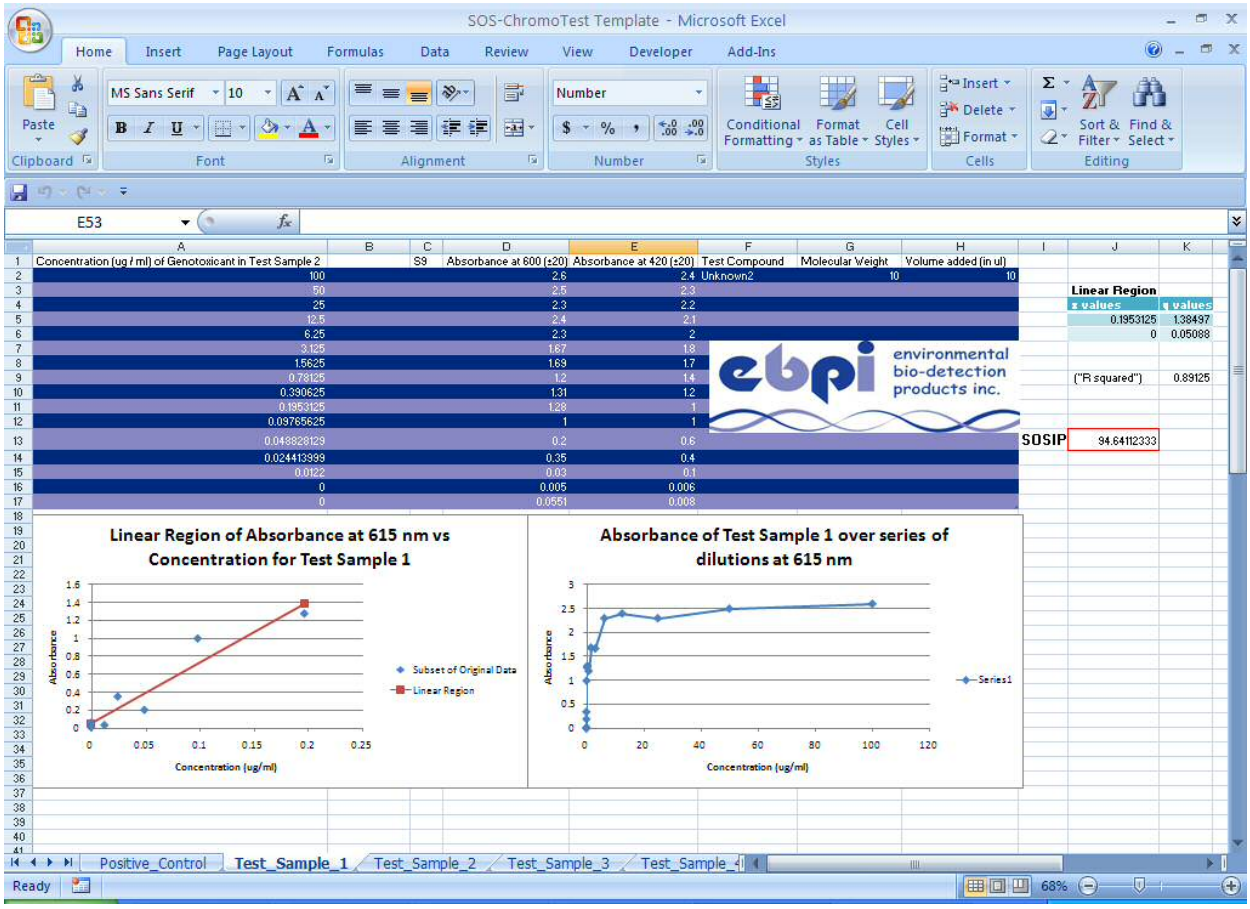

Pictured above is an example of how the Positive Control worksheet should appear after data entry. You will notice that there is also a small table to the right of the one where you have entered concentration and absorbencies. This table will display the points used for the linear region of the absorbance vs concentration graph, as shown in Figure 1 of the Instruction Manual accompaying the kit. Finally, there is also an outlined box labelled “SOSIP”. All these values, as well as a graph will be generated once you click on the “Analyze Data” button below the data you entered.




Data for test samples is entered identically to the positive control. Simply enter the concentrations and the absorbance for each concentration at both 600 (±20) nm and 420 (±20) nm as well as the molecular weight and volume of sample added. If these values are not provided, the analysis will be incomplete and an SOSIP will not be calculated for that sample.

You will notice that a table for the coordinates of the linear region of the absorbance vs concentration graph is provided to the right of the raw data. As well, the coefficient of determination ($R^2$value) is also displayed to give an idea of the fit of this line. Finally, the SOSIP is also displayed in the box outlined in red.

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




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 the experiment. Once you have saved or cliekced on “Cancel”, the spreadsheet will analyze the data, giving numerical and graphical results.

Pictured above are the results for a test sample. You can see that two graphs are generated, one being simply the concentratoin vs absorabance for the sample over the entire dilution range, as well as a second graph showing the linear region with the calculated line of best fit. As described previously, the points used for the line of best fit are presented in the table to the right of the raw data, with the accompaying coefficient of determination. The SOSIP is also reported in the red outlined box.

If you are not satisfied with the results of the macro, for whatever reason (ie if you would like to linear region to span a lesser number of points), you may either change

the minimum coefficient of determation found on the Positive Control worksheet, or simply forego the use of the macro and analyze the results in a separate column, using the methods described in the instruction manual accompaying the kit and the raw data already entered. The macro uses an algorithm which calculates the highest possible SOSIP given the limitation of the minimum coefficient of determination. If, for some reason, your data does not conform to the expected curve, then you will have to use your discretion in interpreting results, as well as the validity of that particular sample.

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@biotoxicity.com or by phone at 905-487-7359.