

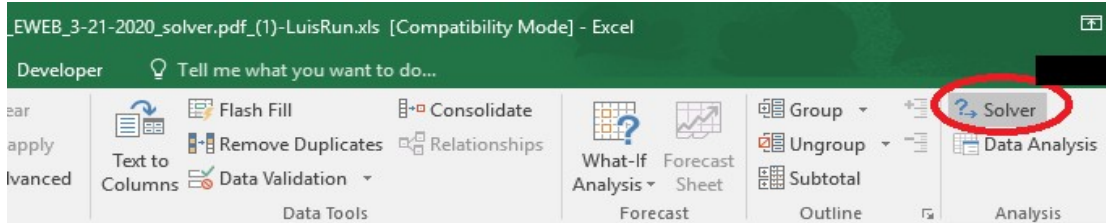
# Gold Standard Diagnostics Solver Instructions



## A. Installing the Solver Add-in

Check that the Solver add-in is enabled in Excel.

If you see a “Solver” button in the Analysis group of the Data tab, then Solver is enabled.



If the Solver is not enabled, you will need to install it (this is a free add-on)

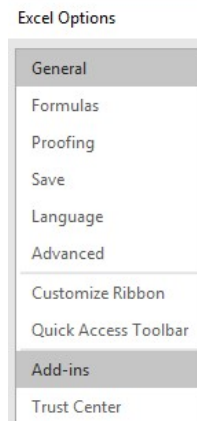
1. Click the file tab on the top left of your screen



2. Select Options (near the bottom of the drop down)



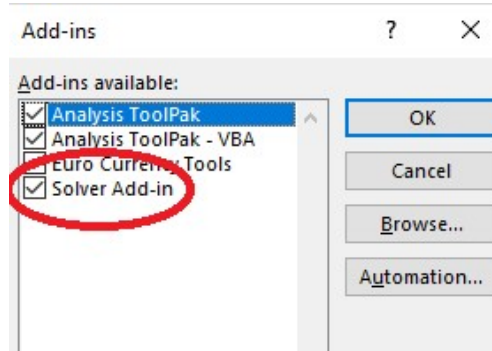
3. A box will generate. Click Add-Ins on left-hand side



4. Once Add-ins is selected, Choose Excel Add-Ins from the drop down option found at the bottom of the window. Then click "Go"



5. Make sure the Solver Add-in is checked, then click "OK"  
The "Solver?" Add-In will install itself. You should now be able to find the icon under the "Data" tab, on the far right-hand side



### B. Using the Solver-Overview

**4-parametric logistic fitting with Excel Solver for CYLINDROSPERMOPSIN ELISA (DUPLICATES)**

**\*\* USER MUST ONLY INPUT VALUES IN GREY-COLORED CELL** Lot Kit #

Std 0	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Amax(0 ppb)	0.05 ppb	0.1 ppb	0.25 ppb	0.5 ppb	1 ppb	2 ppb
1.793	1.562	1.367	1.035	0.772	0.541	0.326
1.821	1.577	1.356	0.987	0.742	0.525	0.328

Graph: OD (averaged) vs Predicted values

CYLINDROSPERMOPSIN (μg)	OD (averaged)	Predicted values	Residual Squares	B/B0	Std Dev	zCV
0.000	1.807	1.813	3.65E-05		0.020	1.096
0.050	1.570	1.551	3.53E-04	0.869	0.011	0.676
0.100	1.362	1.368	4.44E-05	0.753	0.008	0.571
0.250	1.035	1.035	3.70E-05	0.419	0.002	0.607
0.500	0.757	0.751	3.70E-05	0.419	0.021	2.802
1.000	0.533	0.512	4.44E-04	0.295	0.011	2.123
2.000	0.327	0.341	2.03E-04	0.181	0.001	0.432
<b>Sum Of Squares</b>			1.48E-03			

Parameter (Y=(A-D)/(1+(X/C)^B)+D)

Parameter	Value	Units
A	1.81	(Max.)
B	0.96	(Slope)
C	0.30	(IC <sub>50</sub> )
D	0.10	(Min.)

R-Squared: 0.99919

Sample	Absorbance	Std Dev	zCV	CYLINDROSPERMOPSIN (μg) (LINDRO AVG (cell#1))
LRB	1.854			#NUM!
LRB	1.755	0.070	3.879	0.009
QCS	0.662			#NUM!
QCS	0.640	0.016	2.390	0.675
1-1	1.396			0.091
1-1	1.373	0.016	1.175	0.098
4-1	0.840			0.399
4-1	0.813	0.019	2.310	0.427
1-2	1.417			0.085
1-2	1.383	0.024	1.717	0.095
4-2	0.844			0.395
4-2	0.882	0.027	3.114	0.359
1-3	1.393			0.092
1-3	1.376	0.012	0.868	0.098
4-3	0.811			0.429
4-3	0.826	0.011	1.296	0.413
1-4	1.400			0.090
1-4	1.384	0.011	0.813	0.095

Enter Standard Absorbance Values Here



Graph



Enter Standard Concentration Values Here



Enter Control and Sample Names along with their Absorbance Values Here



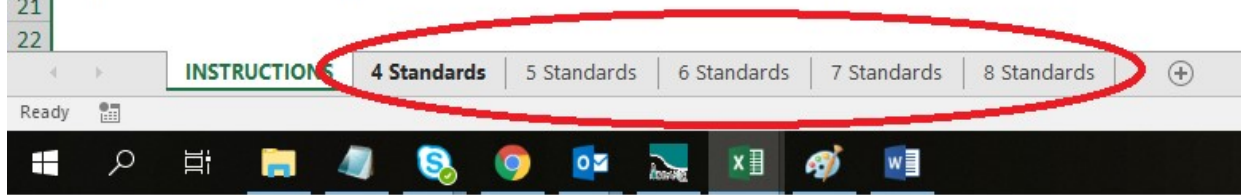
Results will be generated here. Be sure to hit Solve!



## C. Using the Solver-Step by Step

1. If you are using the general solver, it will have tabs that correlate to the number of standards in the kit. Start by selecting the tab that contains the number of standards in your assay.

19 If for some reason the Solver function gives an error, please estimate the absorbance (OD) value at the mid-point of  
 20 proposed curve, then enter your estimation into the yellow box denoted "C" (IC50). Re-solve the equation.  
 21  
 22



\*\*\*\*\*If you are using an assay specific solver, there will only be one tab to select.

2. Next, you will need to enter the concentrations of your standards. You can find the correct standard concentrations in the user's guide under the Materials Provided section.

### A. Materials Provided

1. Microtiter plate (12 X 8 strips) coated with an analog of Microcystins conjugated to a protein
2. Standards (6): 0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb, 1 mL each
3. Control:  $0.75 \pm 0.185$  ppb, 1 mL, prepared from a secondary source, for use as a Quality Control Standard (QCS)
4. Sample Diluent, 25 mL, for use as a Laboratory Reagent Blank (LRB) and for dilution of samples above the range of the standard curve
5. Antibody Solution, 6 mL
6. Anti-Sheep-HRP Conjugate Solution, 12 mL
7. Wash Buffer (5X) Concentrate, 100 mL, must be diluted prior to use, see Test Preparation (Section E)
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 6 mL

3. Navigate to the chart below the graph. Enter the concentrations into the grey boxes.

Enter the standard concentra			
Standards (ng/ml)	OD (averaged)	Predicted values	
0.000	0.604	0.599	
0.150	0.504	0.515	
0.400	0.447	0.442	
1.000	0.354	0.347	
2.500	0.241	0.241	
5.000	0.163	0.168	
Sum of Squares			

\*\*If you are using a solver specific to your assay, you may skip this step.

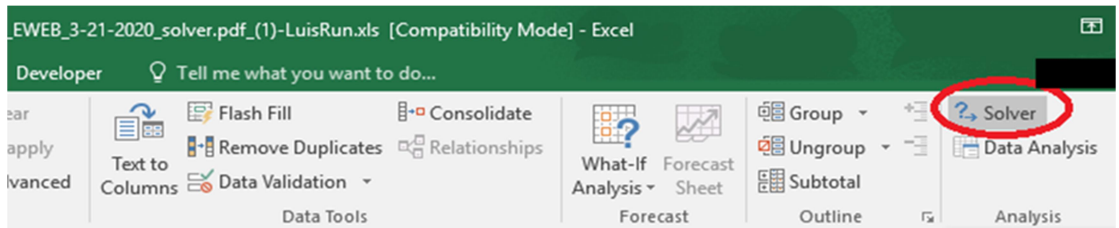
4. Scroll to the top of the spreadsheet and enter the absorbance values of your calibration standards in to the grey boxes.

Enter the absorbance values of each standard into the grey boxes					
Std 0	Std 1	Std 2	Std 3	Std 4	Std 5
0.639	0.499	0.442	0.345	0.241	0.162
0.568	0.508	0.451	0.363	0.241	0.163

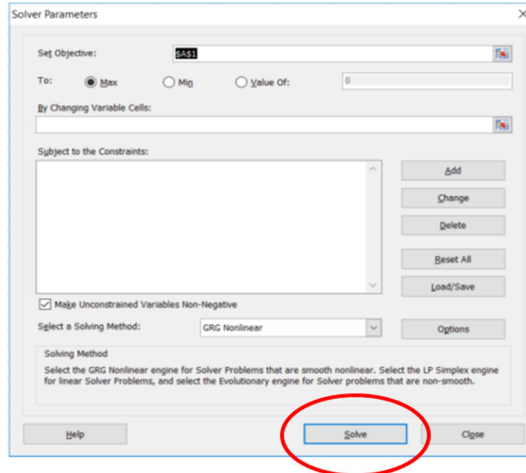
5. Enter the names of your controls and samples along with their absorbance values into the grey boxes in the chart at the bottom of the sheet.

Enter descri	
Sample/Control	Absorbances
Control	0.381
Control	0.389
LRB	0.583
LRB	0.623

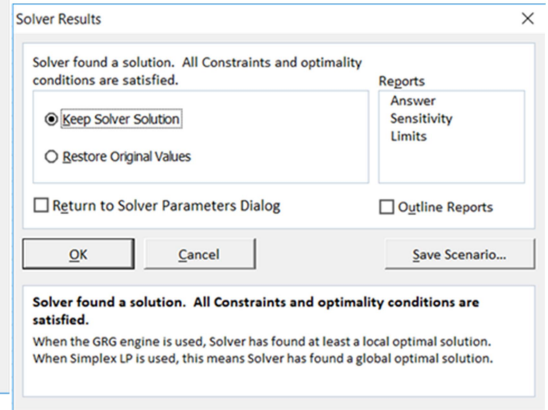
6. Once the data is entered, navigate to the data tab and click the Solver button and click Solve and then OK.



7. A box will generate. Click "Solve"



8. Once the calculations are made, a second box will generate. Make sure "Keep Solver Solution" is selected, then click "OK"



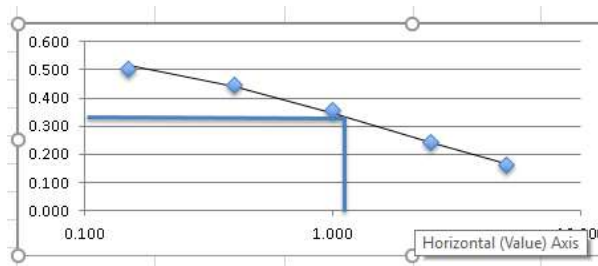
### D. Troubleshooting

Sometimes the line of best fit will either be a horizontal, flat line or it will not be close to any of the calibration points. This is fairly common and fairly easy to troubleshoot. The first thing you can try is to enter 1 into all the 4 yellow boxes next to the equation components below the graph, then clicking Solve to solve the equation of the line as described in the previous paragraph.

Parameter  $(Y=(A-D)/(1+(X/C)^B)+D)$

A	1.000	(Max.)
B	1.000	(Slope)
C	1.000	(IC <sub>50</sub> )
D	1.000	(Min.)

If this does not work, try estimating where the line of best fit would intersect with the Y axis and enter that corresponds to it in the yellow box next to the C component and try solving the solver again.



In this example above, the graph of the line would intersect the Y axis at approximately 0.32 (as demonstrated by the horizontal line). The value on the X axis that corresponds to this value is approximately 1.5, which is the number that would go in the yellow box next to C below the graph in the solver.

If you try both of these suggestions and are still having issues, please send a copy of your solver to [support.abraxaxis@us.goldstandarddiagnostics..](mailto:support.abraxaxis@us.goldstandarddiagnostics..)