# **KRISHGEN BioSystems** OUR REAGENTS, YOUR RESEARCH

# **Online ELISA Calculator Instructions and Guide**

1. In the first section as below, first click on the "Clear Signal Input" to remove any existing data that may be there due to cookies. Then, add your absorbance data readings. You can do this by typing in the results or copy-and-pasting from another document.

#### online ELISA assay results calculator

Perform online curve fitting and calculation of your ELISA assay data using this page using AssayFit Pro version 5.3.1 You can use this service for free 15 times each day. Click here to read the terms for use and distribution.

Alternatively use the Microsoft Excel add-in to perform the calculation. Previous version 5.2 Check the menu for other online options and previous versions. For Research Use Only. Not for use in diagnostic procedures.

#### Paste ELISA results here

Enter your ELISA data in the spreadsheet like table below, you can copy and paste from Excel. Optional: you can subtract blank values or normalize, select this from the options menu.

	1	2	3	4	5	6	7	8	9	10	11	12
А	2.657	2.624	0	2.64	0.104	0.115						
В	1.879	1.875	2	1.95								
С	1.023	1.032	1.6	1.61								
D	0.557	0.557	1.2	1.21								
E	0.275	0.265	0.8	0.81								
F	0.191	0.191	0.4	0.41								
G	0.142	0.138	0.2	0.21								
н	0.104	0.104	0.15	0.16								
Clea	ar signal inpu	t	0	ptions 👻	7							
	-											

2. In the section below, add your standard / calibrator labels and sample ID. This indicates to the software what your plate layout is. You can also import the data directly from a .csv format file.

The Toggle Calibrator button allows you to change CAL-X to just the value and vice versa.

ELISA	LISA layout and sample IDs													
Select a buttons sent ove	elect a region and click the B, C, S, CAL buttons to fill the region with blank, control, sample or calibrator values. You can use the S>, Rv uttons to specify the direction of the filling process and the number of replicates. Sample IDs are kept on your local computer and are not ent over the internet.													
	1	2	3	4	5	6	7	8	9	10	11	12		
Α	A CAL-640 CAL-640 Blank Control Sample 8 Sample 8 Control													
в	B CAL-320 CAL-320 Sample 1 Sample 1 Sample 1													
С	C CAL-160 CAL-160 Sample 2 Sample 2 Sample 2 CAL-160 CA													
D	D CAL-80 CAL-80 Sample 3 Sample 3													
E	E CAL-40 Sample 4 Sample 4													
F	CAL-20	CAL-20	Sample 5	Sample 5										
G	CAL-10	CAL-10	Sample 6	Sample 6										
н	CAL-0	CAL-0	Sample 7	Sample 7										
Тод	Toggle calibrators Import IDs from CS\ X B C S CAL Sv R> 2													

Sample dilution А В С D Е F G н Cals from layout Options -

3. In the Sample Dilution section, add in the sample dilution factor that you want to apply for the final results received. This will allow the calculator to automatically apply the DF multiplication.

4. Add the calibrator information in the below section. Copy the values from Excel, enter them manually or use the 'Cals from layout' button to copy all calibrator values marked with CAL- from the layout.

Curve fit input values															
You can from the	You can copy the values from Excel, enter them manually or use the 'Cals from layout' button to copy all calibrator values marked with CAL- rom the layout.														
	Id     weight     Conc     Abs 1     Abs 2     Abs 3     Abs 4     Abs 5     Abs 6     Abs 7     Abs 8     Abs 9     Abs 10														
1	CAL-640	1.0	640.000	2.641											
2	CAL-320	1.0	320.000	1.877											
3	CAL-160	1.0	160.000	1.028											
4	CAL-80	1.0	80.000	0.557											
5	CAL-40	1.0	40.000	0.270											
6	CAL-20	1.0	20.000	0.191											
7	CAL-10	1.0	10.000	0.140											
8	CAL-0	1.0	0.000	0.104											
9															
10															

5. Select the relevant curve fit settings (usually 4PL, but we request you to cross check against the datasheet that accompanied your kit.

Curve fit settings				
4PL	~	Function	Run 1	Run ID
server 1 (EU berlin)	~	Server	Submit	
free		Кеу		

# **ELISA Data Analysis Results Page**

6. On the results page, the following information is seen:



You can toggle your settings and export relevant information. You can even cross-check the input information.

## **Fit Summary**

#### **Calibration curve**

arameter	Value	Info		ld	weight	Conc	Abs mean
Α	3.52663479613	Function	(D + ((A - D) / (1 + ((x / C) ^ B))))  4 parameter logistic	CAL-640	1.0	640.000	2.641
в	-1.45689454311	Run ID	Run1	CAL-320	1.0	320.000	1.877
с	310.25539527500	User		CAL-160	1.0	160.000	1.028
D	0.11618779293	Time	1/29/2024 10:51:08 AM	CAL-80	1.0	80.000	0.557
E				CAL-40	1.0	40.000	0.270
SSq	0.00222977098	R2	0.99984100987	CAL-20	1.0	20.000	0.191
				CAL-10	1.0	10.000	0.140
				CAL-0	1.0	0.000	0.104

## **Complete Results**

ID	Abs 1	Abs 2	Abs 3	Abs mean	Abs CV	Ν	ID	Conc 1	Conc 2	Conc 3	Conc mean	Dilution	Conc x dilu	Conc CV	Position	
CAL-0	0.104	0.104		0.104	0.0	2	CAL-0	0.0	0.0		0.0	2.0	0.0		H2,H1	
CAL-10	0.138	0.142		0.140	2.0	2	CAL-10	9.7	10.9		10.3	2.0	20.6	8.2	G2,G1	
CAL-20	0.191	0.191		0.191	0.0	2	CAL-20	22.9	22.9		22.9	2.0	45.8	0.0	F2,F1	
CAL-40	0.265	0.275		0.270	2.6	2	CAL-40	37.3	39.1		38.2	2.0	76.3	3.3	E2,E1	
CAL-80	0.557	0.557		0.557	0.0	2	CAL-80	83.8	83.8		83.8	2.0	167.5	0.0	D2,D1	
CAL-160	1.032	1.023		1.028	0.6	2	CAL-160	156.0	154.5		155.2	2.0	310.5	0.7	C2,C1	
CAL-320	1.875	1.879		1.877	0.2	2	CAL-320	323.9	325.0		324.5	2.0	648.9	0.2	B2,B1	
CAL-640	2.624	2.657		2.641	0.9	2	CAL-640	625.6	647.6		636.6	2.0	1,273.3	2.4	A2,A1	
Sample 1	1.950	2.000		1.975	1.8	2	Sample 1	344.2	358.4		351.3	various	523.4	2.9	B4,B3	
Sample 2	1.600	1.610		1.605	0.4	2	Sample 2	259.3	261.5		260.4	1.0	260.4	0.6	C3,C4	
Sample 3	1.200	1.210		1.205	0.6	2	Sample 3	183.7	185.4		184.5	various	277.2	0.7	D3,D4	
Sample 4	0.800	0.810		0.805	0.9	2	Sample 4	120.1	121.6		120.8	various	180.8	0.9	E3,E4	
Sample 5	0.400	0.410		0.405	1.7	2	Sample 5	59.8	61.3		60.6	1.0	60.6	1.8	F3,F4	
Sample 6	0.200	0.210		0.205	3.4	2	Sample 6	24.8	26.8		25.8	1.0	25.8	5.6	G3,G4	
Sample 7	0.150	0.160		0.155	4.6	2	Sample 7	13.2	15.8		14.5	1.0	14.5	12.7	H3,H4	
Sample 8	0.104	0.115		0.110	7.1	2	Sample 8	0.0	0.0		0.0	1.0	0.0		A5,A6	
Control	2.640			2.640		1	Control	636.1			636.1	1.0	636.1		A4	

## Sample ID

	1	2	3	4	5	6	7	8	9	10	11	12
Α	CAL-640	CAL-640	Blank	Control	Sample 8	Sample 8						
В	CAL-320	CAL-320	Sample 1	Sample 1								
С	CAL-160	CAL-160	Sample 2	Sample 2								
D	CAL-80	CAL-80	Sample 3	Sample 3								
E	CAL-40	CAL-40	Sample 4	Sample 4								
F	CAL-20	CAL-20	Sample 5	Sample 5								
G	CAL-10	CAL-10	Sample 6	Sample 6								
н	CAL-0	CAL-0	Sample 7	Sample 7								

## Response

	1	2	3	4	5	6	7	8	9	10	11	12
Α	2.657	2.624		2.64	0.104	0.115						
в	1.879	1.875	2	1.95								
С	1.023	1.032	1.6	1.61								
D	0.557	0.557	1.2	1.21								
E	0.275	0.265	0.8	0.81								
F	0.191	0.191	0.4	0.41								
G	0.142	0.138	0.2	0.21								
н	0.104	0.104	0.15	0.16								

### Dilution

	1	2	3	4	5	6	7	8	9	10	11	12
A	2	2	1	1	1	1						
в	2	2	1	2								
С	2	2	1	1								
D	2	2	1	2								
E	2	2	2	1								
F	2	2	1	1								
G	2	2	1	1								
н	2	2	1	1								

The concentration x Dilution results represent the final concentration of your sample, obtained upon multiplying the dilution factor with your interpolated concentration.

## **Conc x Dilution**

	1	2	3	4	5	6	7	8	9	10	11	12
A	1,295.3	1,251.3		636.1	0.0	0.0						
в	650.0	647.9	358.4	688.3								
С	309.0	311.9	259.3	261.5								
D	167.5	167.5	183.7	370.7								
E	78.1	74.5	240.1	121.6								
F	45.8	45.8	59.8	61.3								
G	21.8	19.4	24.8	26.8								
н	0.0	0.0	13.2	15.8								

# Fit output

	xdata	ydata	weights	percent	fittedy	resid	paraminfo	params	yknown	xfromyknowi	xcurve	ycurve	info	infovalue 🔺
1	640.000	2.641	1.000	100.0	2.646	-0.0048	А	3.52663480	2.657	647.631	0.000	0.116	Run ID:	Run1
2	320.000	1.877	1.000	71.1	1.860	0.0172	в	-1.45689454	1.879	324.984	0.000	0.116		
3	160.000	1.028	1.000	38.9	1.057	-0.0292	С	310.2553952	1.023	154.520	0.000	0.116	Function:	401
4	80.000	0.557	1.000	21.1	0.532	0.0251	D	0.11618779	0.557	83.768	0.000	0.116		1.21
5	40.000	0.270	1.000	10.2	0.280	-0.0103			0.275	39.058	0.000	0.116	UTC DateTime:	1/29/2024 10:51:0
6	20.000	0.191	1.000	7.2	0.178	0.0131			0.191	22.894	0.000	0.116		
7	10.000	0.140	1.000	5.3	0.139	0.0011	Fit function:		0.142	10.918	0.000	0.116	User:	****
8	0.000	0.104	1.000	3.9	0.116	-0.0122	(D + ((A - D) / (1 +		0.104	0.000	0.000	0.116		
							((x / C) ^ B))))  4							
							parameter logistic							
9									2.624	625.645	0.000	0.116	IP address:	****

Get in touch with our team at <u>info@krishgen.com</u> for support and visit our website <u>www.krishgen.biz</u> for more information on our ELISA kits.