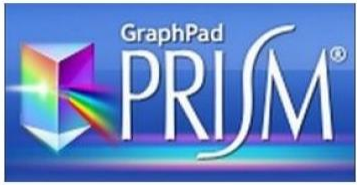


How to analyze data in GraphPad Prism

Chonticha Areebambud

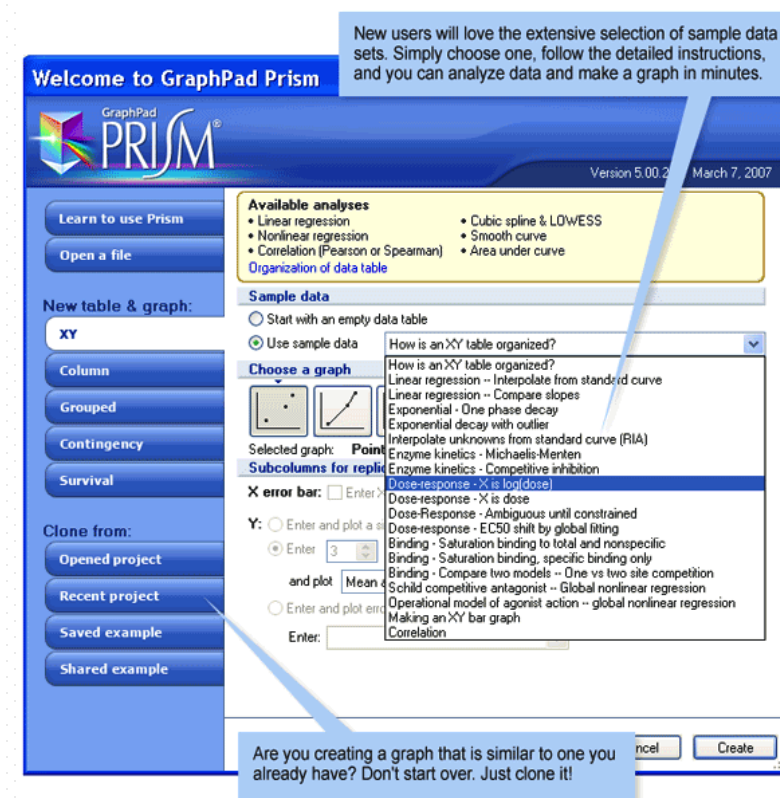
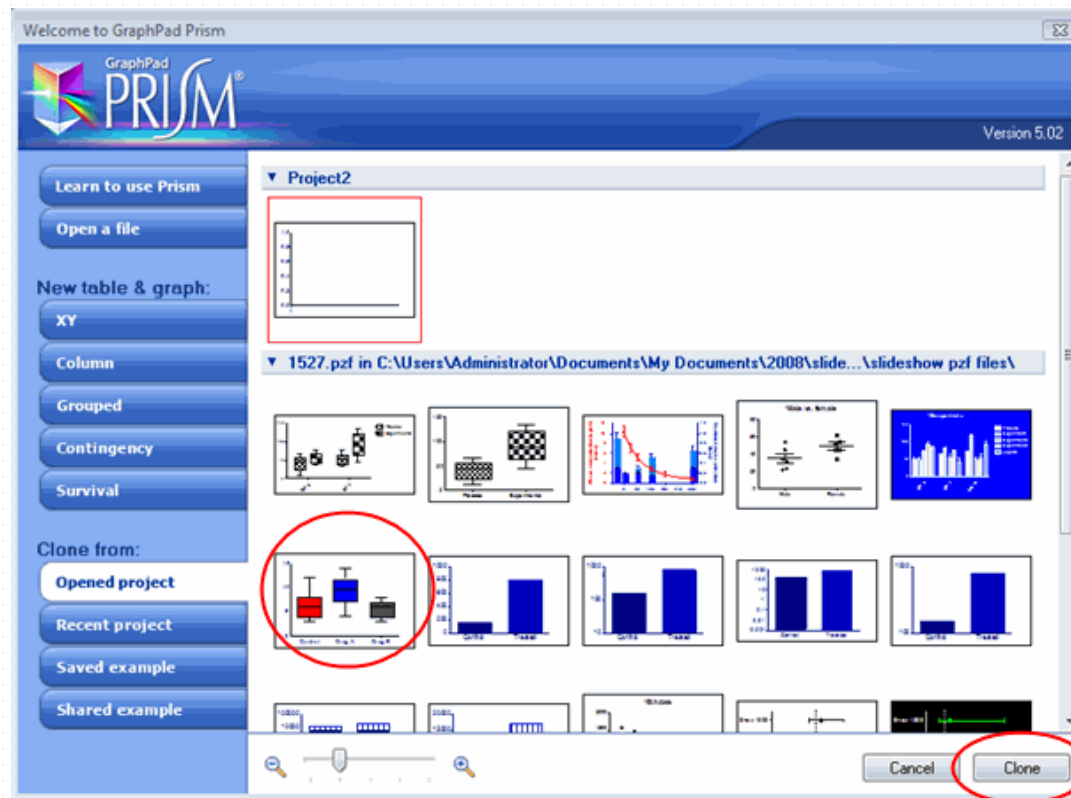
Academic Officer,
ThaiHealth Academy,
Thai Health Promotion Foundation

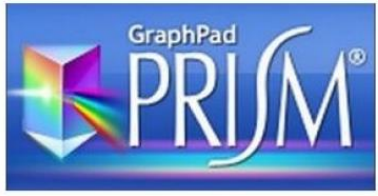
26 June 2024



What is GraphPad Prism?

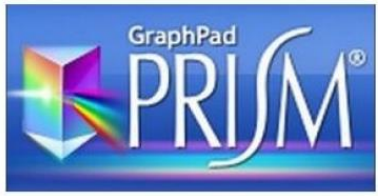
➤ GraphPad Prism is the data analysis and visualization software for scientific research.





What is GraphPad Prism?

- Combines nonlinear regression (curve fitting), basic biostatistics, and scientific graphing
- Help you efficiently analyze, graph, and organize your experimental data. Notable features include:
 - Automatic error bars
 - Easy curve fitting. Fit curves in one step.
 - Automate routine analyses.
 - A complete record of your work. Prism stores all parts of an experiment (data, results, graphs, page layouts, and notes) in one organized file.

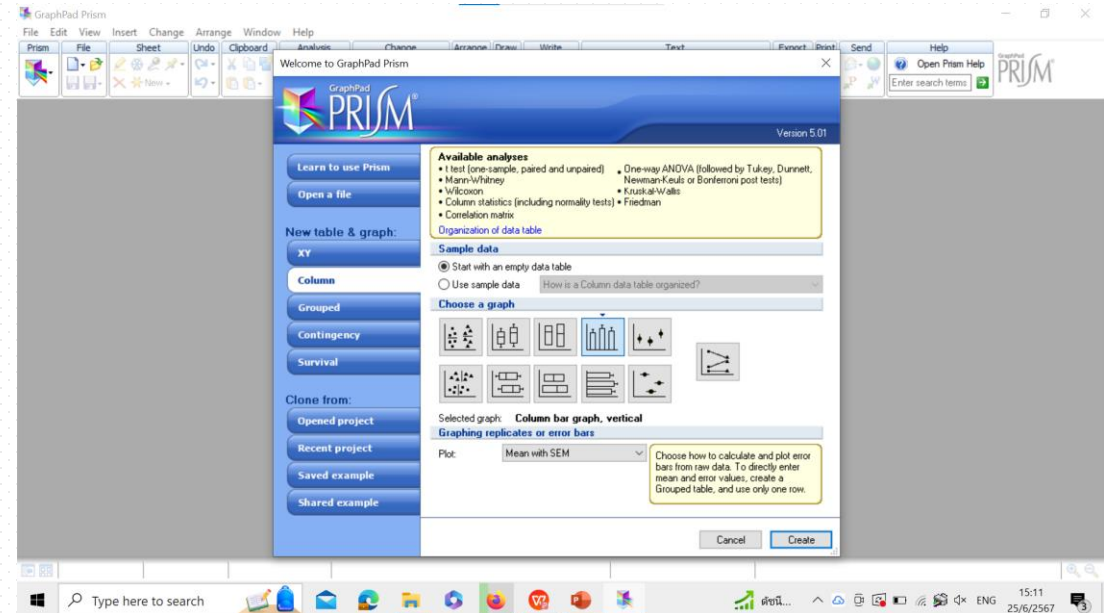


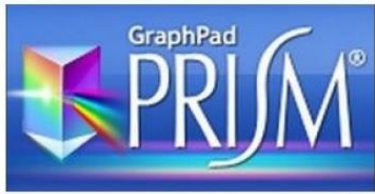
How to use the GraphPad Prism?

Step 1. Start Prism

- Data
- Graph types
- Analyses from a one-way table:
 - t-test (one-sample, paired and unpaired)
 - One-way ANOVA (followed by Tukey, Dunnett, Newman-Keuls, or Bonferroni post-tests)

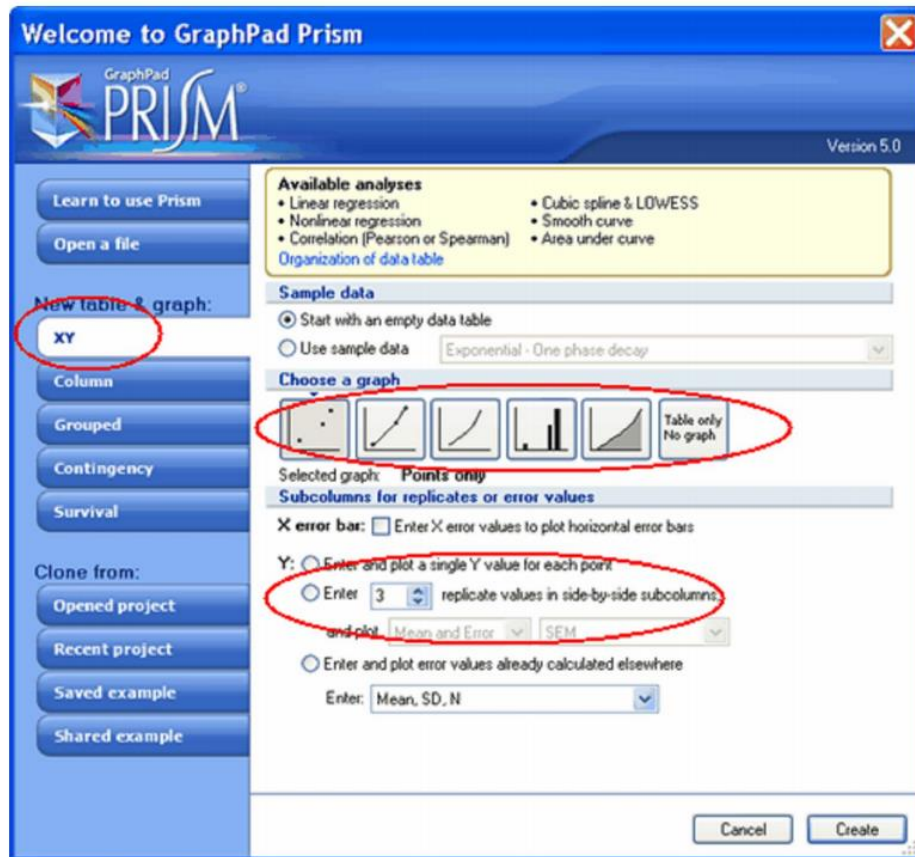
No.	FBG (mg/dL)			
	CON	DM2	DM2-ML	DM2-MP
1	92	330	225	165
2	89	328	230	160
3	92	310	262	164
4	91	350	234	174

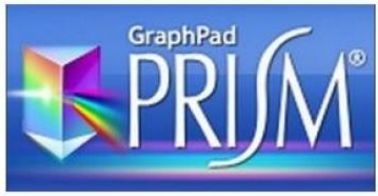




How to use the GraphPad Prism?

Step 2. Begin by choosing a graph type





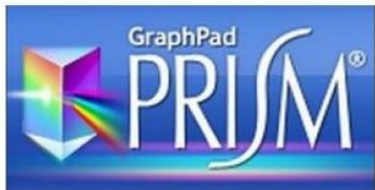
How to use the GraphPad Prism?

Step 3. Enter or import data

The screenshot shows the GraphPad Prism software interface. The main window displays a data table with the following structure:

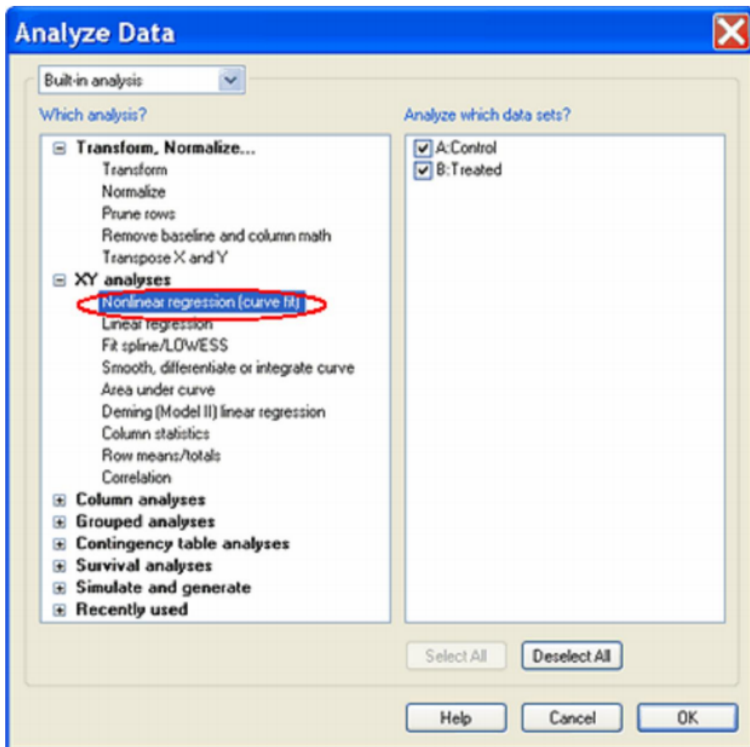
	X	A				B				C			
	Concentration	% Cell viability					Title				Title		
	X	A:Y1	A:Y2	A:Y3	A:Y4	B:Y1	B:Y2	B:Y3	B:Y4	C:Y1	C:Y2	C:Y3	
1	Title	0.00000	100.00	100.00	100.00	100.00							
2	Title	0.15625	95.95	94.91	94.46	96.96							
3	Title	0.31250	90.61	88.73	86.25	86.94							
4	Title	0.62500	83.24	82.91	80.54	83.54							
5	Title	1.25000	47.70	47.64	48.75	47.76							
6	Title	2.50000	44.57	42.18	41.96	42.75							
7	Title												
8	Title												
9	Title												
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18	Title												
19	Title												
20	Title												
21	Title												
22	Title												
23	Title												

The interface includes a menu bar (File, Edit, View, Insert, Change, Arrange, Window, Help), a toolbar with various icons, and a left-hand pane showing a project hierarchy with folders like 'Data with Results', 'Data Tables', 'Info', 'Results', and 'Graphs'. The status bar at the bottom shows 'Data 1' and 'Row CT, X:'.



How to use the GraphPad Prism?

Step 4. Analyze your data



Analyze Data

Built-in analysis: ▼

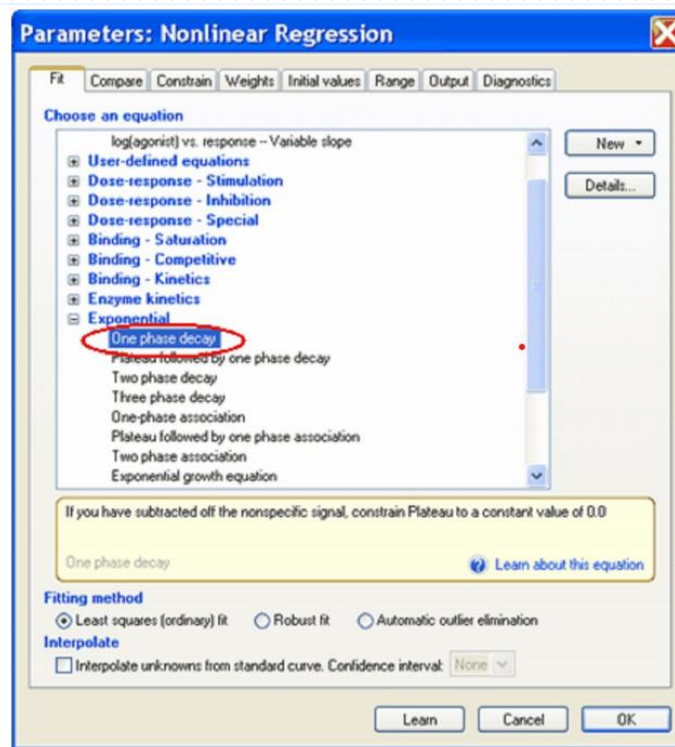
Which analysis? Analyze which data sets?

- Transform, Normalize...
 - Transform
 - Normalize
 - Prune rows
 - Remove baseline and column math
 - Transpose X and Y
- XY analyses**
 - Nonlinear regression (curve fit)**
 - Linear regression
 - Fit spline/LOWESS
 - Smooth, differentiate or integrate curve
 - Area under curve
 - Deming (Model II) linear regression
 - Column statistics
 - Row means/totals
 - Correlation
- Column analyses
- Grouped analyses
- Contingency table analyses
- Survival analyses
- Simulate and generate
- Recently used

A: Control
 B: Treated

Select All Deselect All

Help Cancel OK



Parameters: Nonlinear Regression

Fit Compare Constrain Weights Initial values Range Output Diagnostics

Choose an equation

- log(agonist) vs. response - Variable slope
- User-defined equations
- Dose-response - Stimulation
- Dose-response - Inhibition
- Dose-response - Special
- Binding - Saturation
- Binding - Competitive
- Binding - Kinetics
- Enzyme kinetics
- Exponential**
 - One phase decay**
 - Plateau followed by one phase decay
 - Two phase decay
 - Three phase decay
 - One-phase association
 - Plateau followed by one phase association
 - Two phase association
 - Exponential growth equation

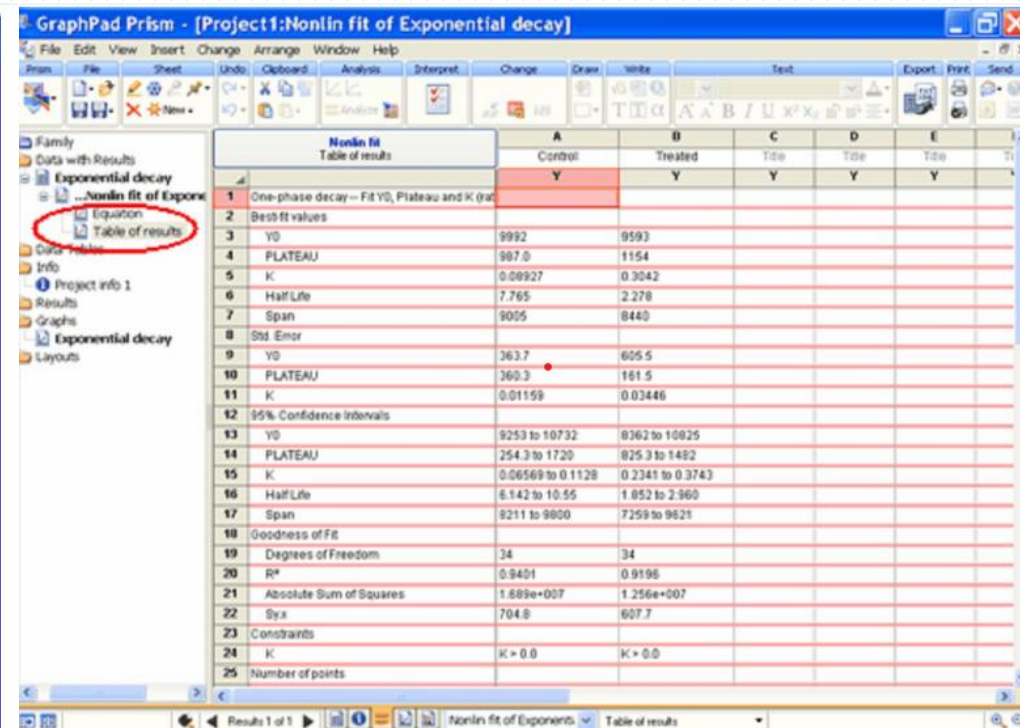
If you have subtracted off the nonspecific signal, constrain Plateau to a constant value of 0.0

One phase decay [Learn about this equation](#)

Fitting method
 Least squares (ordinary) fit Robust fit Automatic outlier elimination

Interpolate
 Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK



GraphPad Prism - [Project1:Nonlin fit of Exponential decay]

File Edit View Insert Sheet Change Arrange Window Help

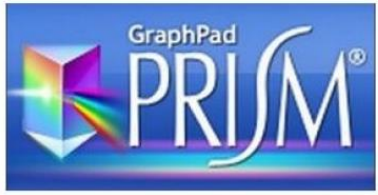
Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send

Family

- Data with Results
- Exponential decay
 - Nonlin fit of Exponential decay
 - Equation
 - Table of results
- Data Tables
- Info
- Project info 1
- Results
- Graphs
- Exponential decay
- Layouts

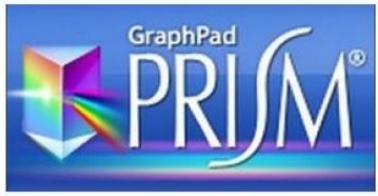
Nonlin fit		A	B	C	D	E
Table of results		Control	Treated	Title	Title	Title
1	One-phase decay - Fit Y0, Plateau and K (quad)	Y	Y	Y	Y	Y
2	Best-fit values					
3	Y0	9992	9593			
4	PLATEAU	987.0	1154			
5	K	0.08927	0.3042			
6	HalfLife	7.765	2.278			
7	Span	9005	8440			
8	Std Error					
9	Y0	263.7	605.5			
10	PLATEAU	260.3	161.5			
11	K	0.01159	0.03446			
12	95% Confidence Intervals					
13	Y0	9253 to 10732	8362 to 10825			
14	PLATEAU	254.3 to 1720	825.3 to 1482			
15	K	0.06569 to 0.1128	0.2341 to 0.3743			
16	HalfLife	6.142 to 10.55	1.852 to 2.960			
17	Span	9211 to 9800	7259 to 9621			
18	Goodness of Fit					
19	Degrees of Freedom	34	34			
20	R ²	0.9401	0.9196			
21	Absolute Sum of Squares	1.689e+007	1.256e+007			
22	Syx	704.8	607.7			
23	Constraints					
24	K	K > 0.0	K > 0.0			
25	Number of points					

Results 1 of 1 Nonlin fit of Exponential decay Table of results



How to use the GraphPad Prism?

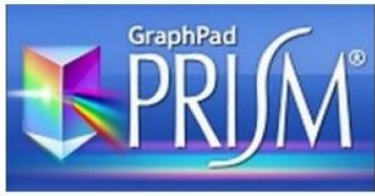
- 1. IC50 Calculation using GraphPad Prism**
- 2. One-way ANOVA**
- 3. Two-way ANOVA**



IC50 Calculation using GraphPad Prism

Step 1. Choose the XY tab, drop the list of sample data sets

The screenshot shows the GraphPad Prism software interface. A dialog box titled "New table & graph:" is open, displaying the "XY" tab. The "Available analyses" section lists: Linear regression, Nonlinear regression, Correlation (Pearson or Spearman), Cubic spline & LOWESS, Smooth curve, and Area under curve. The "Sample data" section has "Start with an empty data table" selected. The "Choose a graph" section shows "Points & connecting line" selected. The "Subcolumns for replicates or error values" section has "Enter and plot a single Y value for each point" selected. The "X error bar" section has "Enter X error values to plot horizontal error bars" selected. The "Y" section has "Enter and plot a single Y value for each point" selected. The "Enter" field is set to "4". The "and plot" dropdown is set to "Mean and Error" and the "SEM" dropdown is set to "SEM". The "Enter" field is set to "Mean, SD, N". The "Create" button is highlighted.



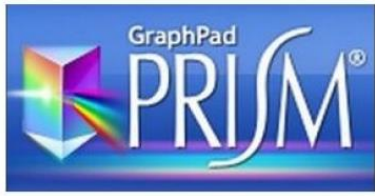
IC50 Calculation using GraphPad Prism

Step 1. Choose the XY tab

The screenshot shows the GraphPad Prism software interface. The 'New table & graph' dialog box is open, and the 'XY' tab is selected. The dialog box contains the following options:

- Available analyses:** Linear regression, Nonlinear regression, Correlation (Pearson or Spearman), Cubic spline & LO/WESS, Smooth curve, Area under curve.
- Sample data:** Start with an empty data table, Use sample data (How is an XY table organized?).
- Choose a graph:** Points & connecting line (Selected graph), Table only, No graph.
- Subcolumns for replicates or error values:** X error bar: Enter X error values to plot horizontal error bars.
- Y:** Enter and plot a single Y value for each point, Enter 4 replicate values in side-by-side subcolumns, and plot Mean and Error SEM, Enter and plot error values already calculated elsewhere (Enter: Mean, SD, N).

The background shows the main GraphPad Prism window with a menu bar (File, Edit, View, Insert, Change, Arrange, Window, Help) and a toolbar. The 'Prism' tab is active, and the 'Data 1' table is visible in the background.



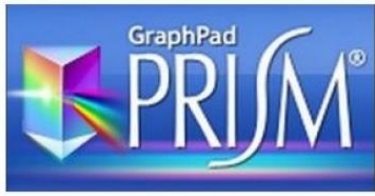
IC50 Calculation using GraphPad Prism

Step 2. Drop the list of sample data sets

The screenshot shows the GraphPad Prism software interface. The main window displays a data table with the following structure:

Table format: XY		A				B				C		
X Title		Title				Title				Title		
	X	A:Y1	A:Y2	A:Y3	A:Y4	B:Y1	B:Y2	B:Y3	B:Y4	C:Y1	C:Y2	C:Y3
1	Title											
2	Title											
3	Title											
4	Title											
5	Title											
6	Title											
7	Title											
8	Title											
9	Title											
10	Title											
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12	Title											
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15	Title											
16	Title											
17	Title											
18	Title											
19	Title											
20	Title											
21	Title											
22	Title											
23	Title											

The interface includes a menu bar (File, Edit, View, Insert, Change, Arrange, Window, Help), a toolbar with various icons, and a left-hand navigation pane showing a project structure with folders for Family, Data Tables, Info, Results, Graphs, and Layouts. The status bar at the bottom indicates the current row and column (Row 1, Column X) and the system tray shows the time (16:15) and date (25/6/2567).



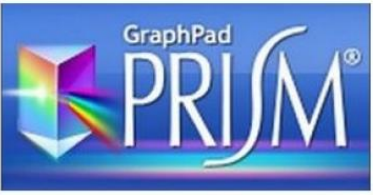
IC50 Calculation using GraphPad Prism

Step 2. Drop the list of sample data sets

The screenshot shows the GraphPad Prism software interface. The main window displays a data table with the following structure:

	X	A				B				C			
	Concentration	% Cell viability				Title				Title			
	X	A:Y1	A:Y2	A:Y3	A:Y4	B:Y1	B:Y2	B:Y3	B:Y4	C:Y1	C:Y2	C:Y3	C:Y4
1	0.00000	100.00	100.00	100.00	100.00								
2	0.15625	95.95	94.91	94.46	96.96								
3	0.31250	90.61	88.73	86.25	86.94								
4	0.62500	83.24	82.91	80.54	83.54								
5	1.25000	47.70	47.64	48.75	47.76								
6	2.50000	44.57	42.18	41.96	42.75								
7													
8													
9													
10													
11													
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23													

The interface includes a menu bar (File, Edit, View, Insert, Change, Arrange, Window, Help), a toolbar with various icons, and a left-hand navigation pane showing a project structure with folders for Family, Data Tables, Info, Results, Graphs, and Layouts. The status bar at the bottom indicates the current row and column (Row 13, A).



IC50 Calculation using GraphPad Prism

Step 3. Transformation the data

GraphPad Prism - [Project5:Data 1]

File Edit View Insert Change Arrange Window Help

Prism File Sheet Undo Clipboard Analysis Change Import Draw Write Text Export Print Send Help

Analyze Data

Built-in analysis

Which analysis?

- Transform, Normalize...
 - Transform
 - Normalize
 - Prune rows
 - Remove baseline and column math
 - Transpose X and Y
- XY analyses
 - Nonlinear regression (curve fit)
 - Linear regression
 - Fit spline/LOWESS
 - Smooth, differentiate or integrate curve
 - Area under curve
 - Deming (Model II) linear regression
 - Column statistics
 - Row means/totals
 - Correlation
- Column analyses
- Grouped analyses
- Contingency table analyses
- Survival analyses
- Simulate and generate
- Recently used

Analyze which data sets?

A:% Cell viability

When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.

Select All Deselect All

Help Cancel OK

Table format: XY		X
		Concentration
	X	
1	Title	0.00000
2	Title	0.15625
3	Title	0.31250
4	Title	0.62500
5	Title	1.25000
6	Title	2.50000
7	Title	
8	Title	
9	Title	
10	Title	
11	Title	
12	Title	
13	Title	
14	Title	
15	Title	
16	Title	
17	Title	
18	Title	
19	Title	
20	Title	
21	Title	
22	Title	
23	Title	

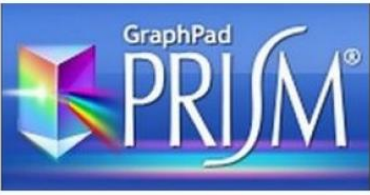
Family

- Data with Results
 - Data 1
 - ...Nonlin fit of Data
 - ...Transform of Data
 -Nonlin fit of Tra
- Data Tables
- Info
 - Project info 1
- Results
- Graphs
 - Data 1
 - Transform of Data 1
- Layouts
- Floating Notes

Row 1, X: Selected: Rows 6, Columns 2

Type here to search

JPY/... 17:06 25/6/2567



IC50 Calculation using GraphPad Prism

Step 4. Analyze the data: Click Nonlinear regression

GraphPad Prism - [Project3:Data 1]

File Edit View Insert Change Arrange Window Help

Prism File Sheet Undo Clipboard Analysis Change Import Draw Write Text Export Print Send Help

Analyze Data

Built-in analysis

Which analysis?

- Transform, Normalize...
 - Transform
 - Normalize
 - Prune rows
 - Remove baseline and column math
 - Transpose X and Y
- XY analyses
 - Nonlinear regression (curve fit)
 - Linear regression**
 - Fit spline/LOWESS
 - Smooth, differentiate or integrate curve
 - Area under curve
 - Deming (Model II) linear regression
 - Column statistics
 - Row means/totals
 - Correlation
- Column analyses
- Grouped analyses
- Contingency table analyses
- Survival analyses
- Simulate and generate
- Recently used

Analyze which data sets?

A:% Cell viability

When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.

Select All Deselect All

Help Cancel OK

	X	A
1	0.00000	
2	0.15625	
3	0.31250	
4	0.62500	
5	1.25000	
6	2.50000	
7		
8		
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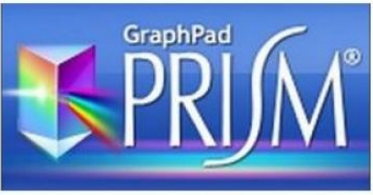
Family

- Data Tables
 - Data 1
- Info
 - Project info 1
- Results
- Graphs
 - Data 1
- Layouts

Row 13, A:

Type here to search

16:28 25/6/2567



IC50 Calculation using GraphPad Prism

Step 5. Choose the "Dose-Response - Inhibition"

GraphPad Prism - [Project3:Data 1]

Parameters: Nonlinear Regression

Fit Compare Constrain Weights Initial values Range Output Diagnostics

Choose an equation

- Recently used
 - One site - Fit logIC50
 - Two sites - Fit logIC50
 - log[inhibitor] vs. normalized response -- Variable slope
- Dose-response - Stimulation**
- Dose-response - Inhibition**
 - log[inhibitor] vs. response
 - log[inhibitor] vs. response -- Variable slope
 - log[inhibitor] vs. normalized response
 - log[inhibitor] vs. normalized response -- Variable slope
- Dose-response - Special
 - Binding - Saturation
 - Binding - Competitive
 - Binding - Kinetics
 - Enzyme kinetics
 - Exponential
 - Lines
 - Polynomial

-If X is not already the log of dose, go back and transform your data.
-The curve will have a standard slope (Hill Slope = -1.0).

log[inhibitor] vs. response [Learn about this equation](#)

Fitting method

Least squares (ordinary) fit Robust fit Automatic outlier elimination

Interpolate

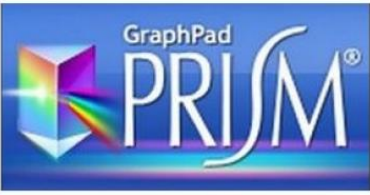
Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK

	X	A
	Concentration	
1	0.00000	
2	0.15625	
3	0.31250	
4	0.62500	
5	1.25000	
6	2.50000	
7		
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23		

Row 13, A:

16:32 25/6/2567



IC50 Calculation using GraphPad Prism

Step 6. Choose "log(inhibitor) vs. response - Variable slope"

The screenshot displays the GraphPad Prism interface with the 'Parameters: Nonlinear Regression' dialog box open. The 'Choose an equation' section is expanded, showing various model categories. Under 'Dose-response - Inhibition', the option 'log(inhibitor) vs. normalized response' is selected. A yellow warning box provides additional information about the equation choice. The background shows a data table with 'Concentration' values and a Windows taskbar at the bottom.

	X	A
	Concentration	
1	0.00000	
2	0.15625	
3	0.31250	
4	0.62500	
5	1.25000	
6	2.50000	
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		

Parameters: Nonlinear Regression

Fit Compare Constrain Weights Initial values Range Output Diagnostics

Choose an equation

- Recently used
 - One site - Fit logIC50
 - Two sites - Fit logIC50
 - log(inhibitor) vs. normalized response -- Variable slope
- Dose-response - Stimulation**
- Dose-response - Inhibition**
 - log(inhibitor) vs. response
 - log(inhibitor) vs. response -- Variable slope
 - log(inhibitor) vs. normalized response**
 - log(inhibitor) vs. normalized response -- Variable slope
- Dose-response - Special**
- Binding - Saturation**
- Binding - Competitive**
- Binding - Kinetics**
- Enzyme kinetics**
- Exponential**
- Lines**
- Polynomial**

-If X is not already the log of dose, go back and transform your data.
-The curve will have a standard slope (Hill Slope = -1.0).
-The Y values of the curve will go from 100% down to 0%.
log(inhibitor) vs. normalized response

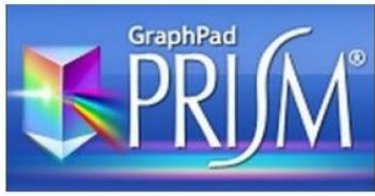
Fitting method

Least squares (ordinary) fit Robust fit Automatic outlier elimination

Interpolate

Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK



IC50 Calculation using GraphPad Prism

Step 7. Click OK and view the results

