



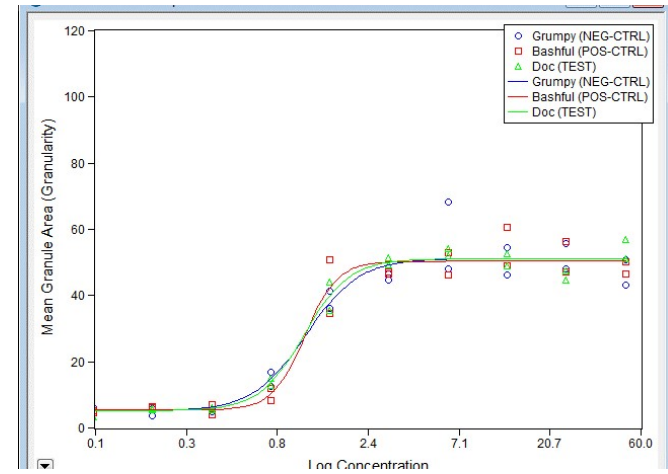
MetaXpress[®] 6.5 Software Guide

Curve Fitting

Date Revised 4/18/2018 Version A



Chapter Purpose



The purpose of this chapter is to introduce the user to the new curve fit feature added in MetaXpress 6.5

This feature requires purchase of the following part:

5058772

CURVE FITTING APPLICATION MODULE.

Includes Peak Pro Analysis.



Review Plate Data

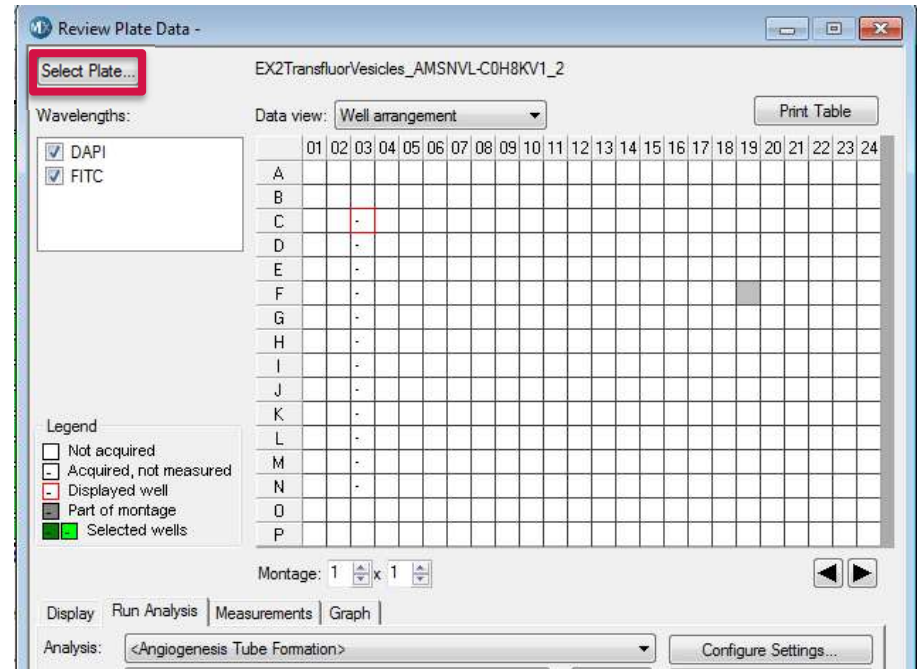
1. Open **Review Plate Data**

- In the main toolbar click on



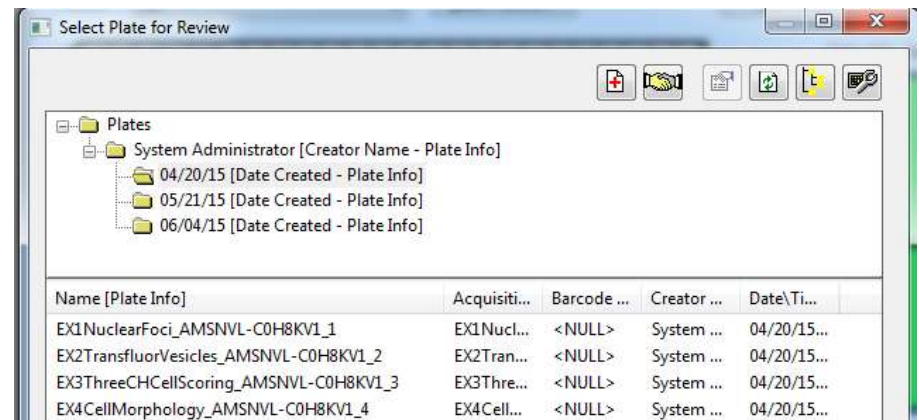
OR

- Under the **Screening** menu, select **Review Plate Data**



2. Click on the **Select Plate** button

3. Browse through the folders to open the plate of interest



Select Measurement Set

- Go to **Review Plate Data > Measurements** tab to select the analysis results of interest

Display | Run Analysis | **Measurements** | Graph

Analysis: Transfluo: Transfluo assay 40 Show Heat Map

Granularity: Granularity for Transfluo assay: 3

Measurement: Transfluo: Transfluo assay 40x: 11 Display Format: ###

Select Wells Based On Variable Range

Value is: Between 0 and 100

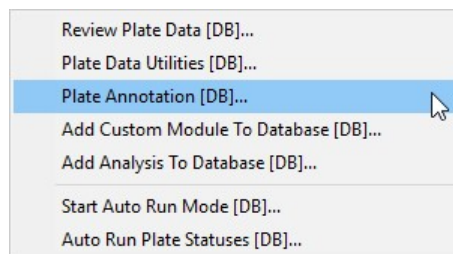
Press "Log Data" button to export the table

Data Log: DDE App



Annotate Plates

- The plate must be annotated for meaningful curve fitting
- If the plate is not already annotated, go to **Screening > Plate Annotation** and add compound and concentration information
- See separate guide on **Plate Annotation in MX or AX** for details



Custom (16 X 24), 1 compound, Dose Response 3 compounds 2 replicates_AMSNVL-69HRFV1_12

Template: Untitled

Current Layout: Dose Response 3 compounds 2 replicates_AMSNVL

A	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM	Undefined 0 µM
B	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
C	Empty	Empty	POS-CTRL 50 µM	POS-CTRL 50 µM	TEST 50 µM	TEST 50 µM	NEG-CTRL 50 µM	NEG-CTRL 50 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
D	Empty	Empty	POS-CTRL 25 µM	POS-CTRL 25 µM	TEST 25 µM	TEST 25 µM	NEG-CTRL 25 µM	NEG-CTRL 25 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	Empty	Empty	POS-CTRL 12.500 µM	POS-CTRL 12.500 µM	TEST 12.500 µM	TEST 12.500 µM	NEG-CTRL 12.500 µM	NEG-CTRL 12.500 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
F	Empty	Empty	POS-CTRL 6.250 µM	POS-CTRL 6.250 µM	TEST 6.250 µM	TEST 6.250 µM	NEG-CTRL 6.250 µM	NEG-CTRL 6.250 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
G	Empty	Empty	POS-CTRL 3.125 µM	POS-CTRL 3.125 µM	TEST 3.125 µM	TEST 3.125 µM	NEG-CTRL 3.125 µM	NEG-CTRL 3.125 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
H	Empty	Empty	POS-CTRL 1.563 µM	POS-CTRL 1.563 µM	TEST 1.563 µM	TEST 1.563 µM	NEG-CTRL 1.563 µM	NEG-CTRL 1.563 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
I	Empty	Empty	POS-CTRL 0.781 µM	POS-CTRL 0.781 µM	TEST 0.781 µM	TEST 0.781 µM	NEG-CTRL 0.781 µM	NEG-CTRL 0.781 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
J	Empty	Empty	POS-CTRL 0.391 µM	POS-CTRL 0.391 µM	TEST 0.391 µM	TEST 0.391 µM	NEG-CTRL 0.391 µM	NEG-CTRL 0.391 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
K	Empty	Empty	POS-CTRL 0.195 µM	POS-CTRL 0.195 µM	TEST 0.195 µM	TEST 0.195 µM	NEG-CTRL 0.195 µM	NEG-CTRL 0.195 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
L	Empty	Empty	POS-CTRL 0.0977 µM	POS-CTRL 0.0977 µM	TEST 0.0977 µM	TEST 0.0977 µM	NEG-CTRL 0.0977 µM	NEG-CTRL 0.0977 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
M	Empty	Empty	POS-CTRL 0.0488 µM	POS-CTRL 0.0488 µM	TEST 0.0488 µM	TEST 0.0488 µM	NEG-CTRL 0.0488 µM	NEG-CTRL 0.0488 µM	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty



Show Graph

- Select **Measurement vs Concentration**
- Select **Measurement** of interest
- Enable or disable specific groups and/or compounds to display
- Click **Show Graph**

Display | Run Analysis | Measurements | Graph

Analysis: Transflur: Transflur assay 40

Graph view: Plate Multiple graphs of displayed wells Single Well

Graph type: Measurement vs Well Row
Histogram
Measurement vs Well Column
Measurement vs Well Row
Measurement vs Well Number
Measurement vs Concentration
Scatter Plot

Measurement: Pit Count (Transflur)

Show Graph Curve Fit...

Display | Run Analysis | Measurements | Graph

Analysis: Transflur: Transflur assay 40

Graph view: Plate Multiple graphs of displayed wells Single Well

Graph type: Measurement vs Concentration

Measurement: Pit Count (Transflur)

Groups: Empty NEG-CTRL POS-CTRL TEST

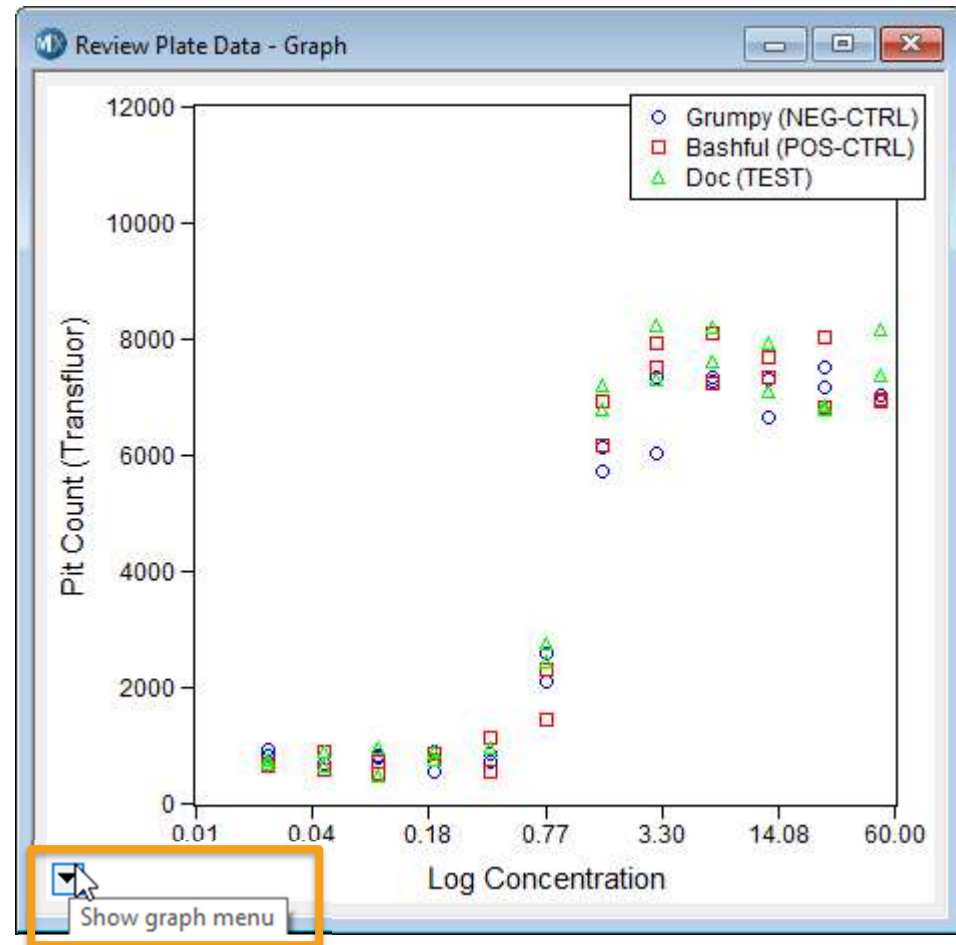
Compounds: Bashful Doc Grumpy Undefined

Show Graph Curve Fit...



Show Graph

- The graph is displayed without any curve fit
- Click on the arrow icon at the lower left to change graph settings, as desired



Curve Fit

- In Review Plate Data > Graph tab, click **Curve Fit**

The screenshot shows the 'Graph' tab of a software interface. The 'Analysis' dropdown is set to 'Transflour: Transflour assay 40'. The 'Graph view' section has three radio buttons: 'Plate' (selected), 'Multiple graphs of displayed wells', and 'Single Well'. The 'Graph type' dropdown is set to 'Measurement vs Concentration'. The 'Measurement' dropdown is set to 'Pit Count (Transflour)'. There are two lists of checkboxes: 'Groups' (Empty, NEG-CTRL, POS-CTRL, TEST) and 'Compounds' (Bashful, Doc, Grumpy, Undefined). Buttons for 'All', 'None', and 'Set Display to Default' are present. The 'Curve Fit...' button is highlighted with an orange box.



Curve Fit Options

- Select **Function**
- **4-Parameter Logistic** is recommended for most dose-response curves
- Select the **Fit Mean** box if you want to use the mean of replicates in the calculation, instead of each individual replicate data point
- Other dose-response options include **5-Parameter Logistic** and **5-Parameter Logistic Alt**

The screenshot shows the 'Curve Fit' dialog box. The 'Function' dropdown is set to '4-Parameter Logistic', and its mathematical equation is displayed as $y = D + \frac{A - D}{1 + (\frac{x}{C})^B}$. The 'Fit Mean' checkbox is unchecked. The 'Compound' dropdown is also set to '4-Parameter Logistic', with 'EC50' set to 1.07127. A table at the bottom lists parameters A, B, C, and D with their respective Error, CI Lower, and CI Upper values.

Parameter	Value	Error	CI Lower	CI Upper
A	Exponential	624	472.126	992.047
B	Bi-exponential	3772	3.54622	6.4823
C	Rectangular Hyperbola	34048	0.959873	1.18267
D	Bi-rectangular Hyperbola	49	7201.84	7721.2



Curve Fit Results

- For **Compound**, select a specific compound to view its curve fit parameters in the table
- If a suitable function is selected, then the **EC50** is displayed for the selected compound.
- Other values such as **EC90** are also available by selecting the appropriate option

Curve Fit

Function: 4-Parameter Logistic $y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$

Fit Mean Weighting: None % Confidence: 95 Asymptotic

Compound: Grumpy (NEG-CTRL) EC50 1.1809

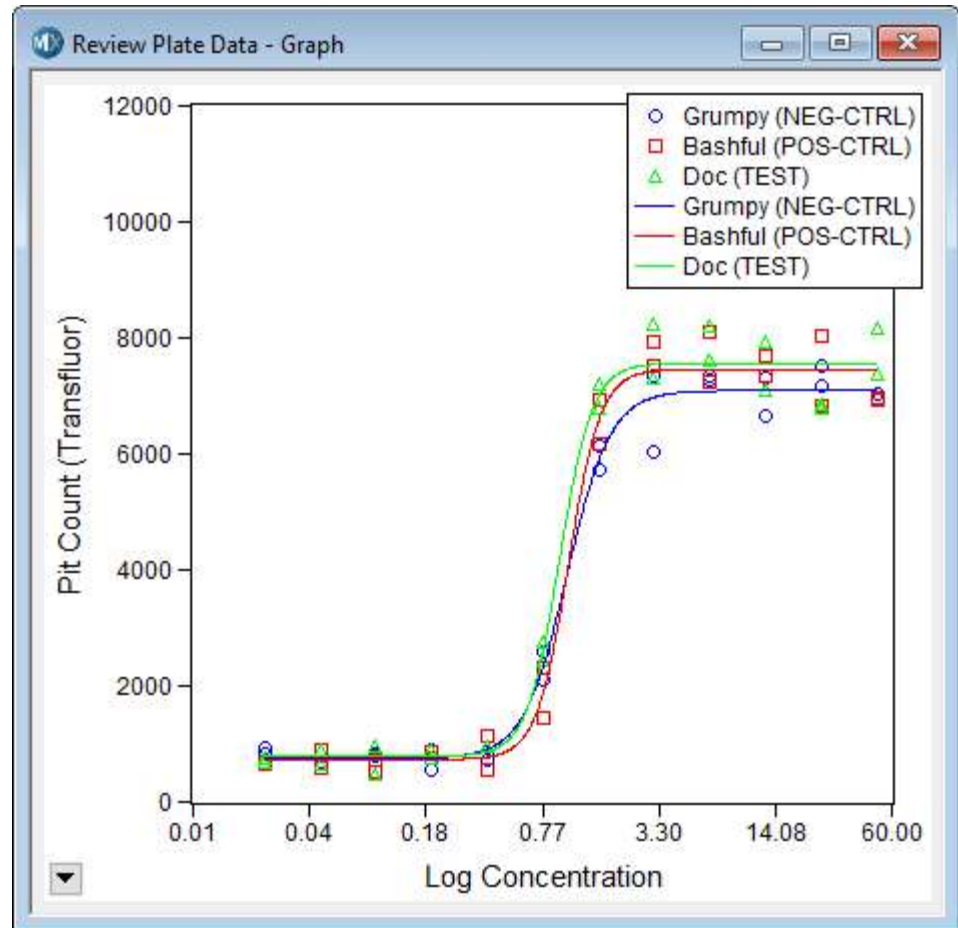
Parameter	Estimate	Std. Error	CI Lower	CI Upper
A	5.04944	1.67542	1.55459	8.5443
B	3.19855	0.823837	1.48006	4.91705
C	1.1809	0.119409	0.931815	1.42998
D	51.2008	1.69888	47.657	54.7446

Configure Log... Open Log BMC... Reset to Default



Curve Fit on Graph

- The selected curve fit is automatically displayed on the graph
- The traces remain on the graph after the Curve Fit window is closed



Log Curve Fit Data

- Click **Configure Log** to select output
- Click **Open Log** to open connection to Excel or a text file (comma-separated format)

Curve Fit

Function: 4-Parameter Logistic $y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$

Fit Mean Weighting: None % Confidence: 95 Asymptotic

Compound: Grumpy (NEG-CTRL) EC50 1.05316

Parameter	Estimate	Std. Error	CI Lower	CI Upper
A	749.787	104.429	531.952	967.621
B	3.68492	0.434628	2.77831	4.59154
C	1.05316	0.0462665	0.95665	1.14967
D	7092.93	103.76	6876.49	7309.36

Configure Log... Open Log BMC... Reset to Default

Open Data Log

Log Measurements to:

Microsoft Excel or other (DDE)

A text file

OK Cancel



Curve Fit output

- It is recommended to either select **Parameter Table** by itself, or select the other outputs without the Parameter Table

Configure Log

Sum-of-Squared Errors
 Degrees of Freedom
 R-Squared
 EC50
 Parameter Table

OK Cancel

Dose Response 3 compounds 2 replicates_AMSNVL-69HRFV1_12	Pit Count (Transflour)	4-Parameter Logistic	Weighting: None	Using Replicates	Asymptotic 95% Confidence Intervals
Data Set	Sum-of-Squared Errors	Degrees of Freedom	R-Squared	EC50	
Grumpy (NEG-CTRL)	1976473.682	20	0.9906876	1.053160693	
Bashful (POS-CTRL)	3008443.174	20	0.9879941	1.071273317	
Doc (TEST)	3078562.186	20	0.987772225	0.958279803	

Configure Log

Sum-of-Squared Errors
 Degrees of Freedom
 R-Squared
 EC50
 Parameter Table

OK Cancel

Dose Response 3 compounds 2 replicates_AMSNVL-69HRFV1_12	Pit Count (Transflour)	4-Parameter Logistic	Weighting: None	Using Replicates	Asymptotic 95% Confidence Intervals
Data Set					
Grumpy (NEG-CTRL)					
Parameter	Estimate	Std. Error	CI Lower	CI Upper	
A	749.7868162	104.4287046	531.9523555	967.6212768	
B	3.684923411	0.434627766	2.778305778	4.591541045	
C	1.053160693	0.046266533	0.956650397	1.149670988	
D	7092.925238	103.759732	6876.48623	7309.364247	
Bashful (POS-CTRL)					
Parameter	Estimate	Std. Error	CI Lower	CI Upper	
A	732.0864975	124.6236111	472.1262002	992.0467948	
B	5.01425968	0.70377181	3.546217409	6.482301951	
C	1.071273317	0.053404793	0.959872871	1.182673763	
D	7461.52055	124.4897445	7201.839494	7721.201607	
Doc (TEST)					
Parameter	Estimate	Std. Error	CI Lower	CI Upper	
A	787.6036641	126.8357112	523.0290069	1052.178321	
B	4.909111636	0.881680205	3.069958956	6.748264316	
C	0.958279803	0.046476699	0.861331109	1.055228497	
D	7562.876421	125.7946	7300.473483	7825.279358	



Curve Fit Function Visual Guide

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- **No Fit - default**
- Linear
- Quadratic
- Cubic
- Quartic
- Semi-Log
- Log-Log
- 2-Parameter Exponential
- 4-Parameter Logistic
- 5-Parameter Logistic
- 5-Parameter Logistic Alternate
- Brain Cousens
- Exponential
- Bi-Exponential
- Rectangular Hyperbola
- RHPlusLinear
- Bi-rectangular Hyperbola
- Two-Site Competition
- Gaussian



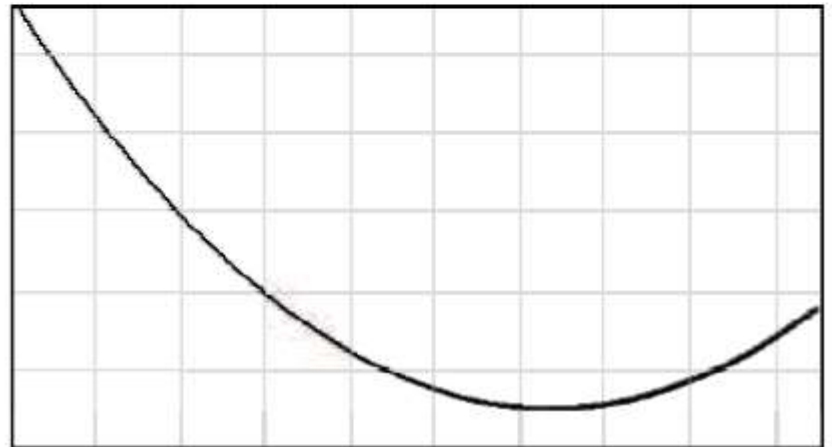
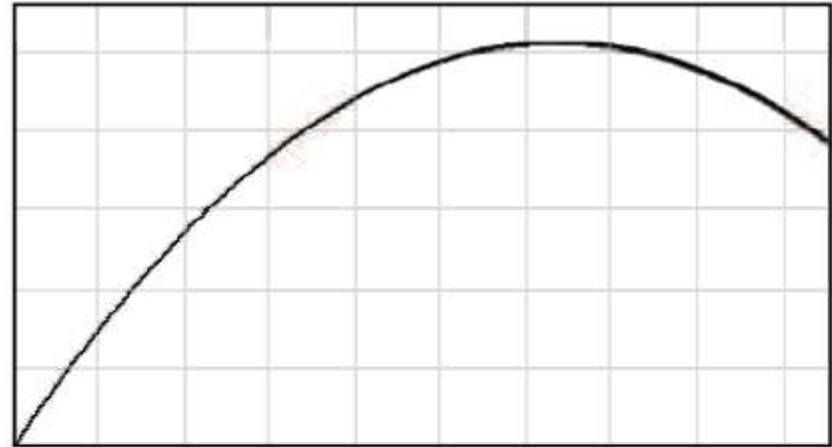
Linear

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- **Linear**
- Quadratic
- Cubic
- Quartic
- Semi-Log
- Log-Log
- 2-Parameter Exponential
- 4-Parameter Logistic
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- 5-Parameter Logistic Alternate
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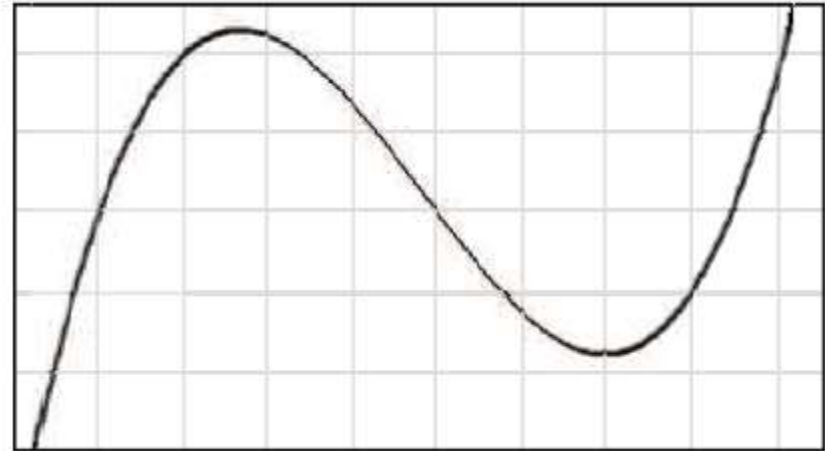
Quadratic

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- Linear
- **Quadratic**
- Cubic
- Quartic
- Semi-Log
- Log-Log
- 2-Parameter Exponential
- 4-Parameter Logistic
- 5-Parameter Logistic
- 5-Parameter Logistic Alternate
- Brain Cousens
- Exponential
- Bi-Exponential
- Rectangular Hyperbola
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- Bi-rectangular Hyperbola
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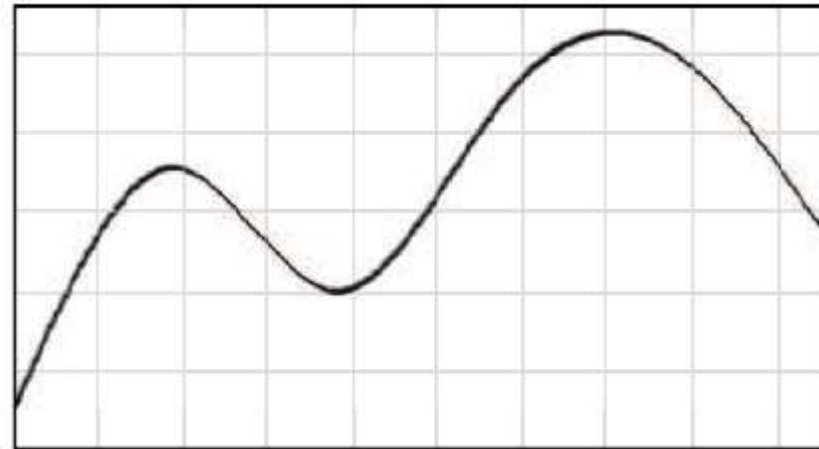
Cubic

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- Linear
- Quadratic
- **Cubic**
- Quartic
- Semi-Log
- Log-Log
- 2-Parameter Exponential
- 4-Parameter Logistic
- 5-Parameter Logistic
- 5-Parameter Logistic Alternate
- Brain Cousens
- Exponential
- Bi-Exponential
- Rectangular Hyperbola
- RHPlusLinear
- Bi-rectangular Hyperbola
- Two-Site Competition
- Gaussian



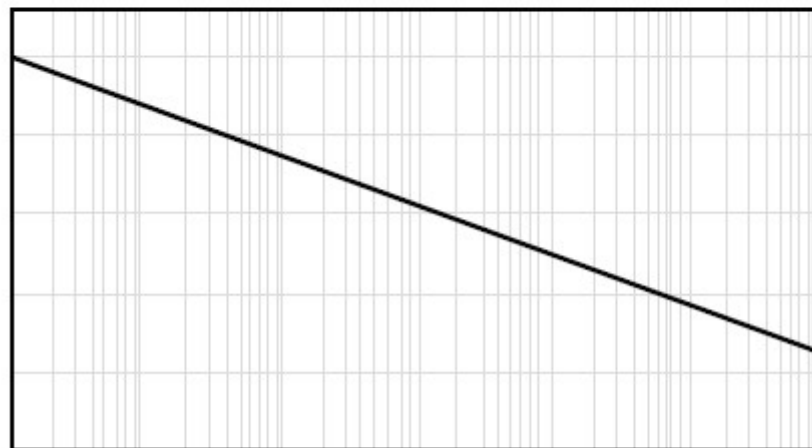
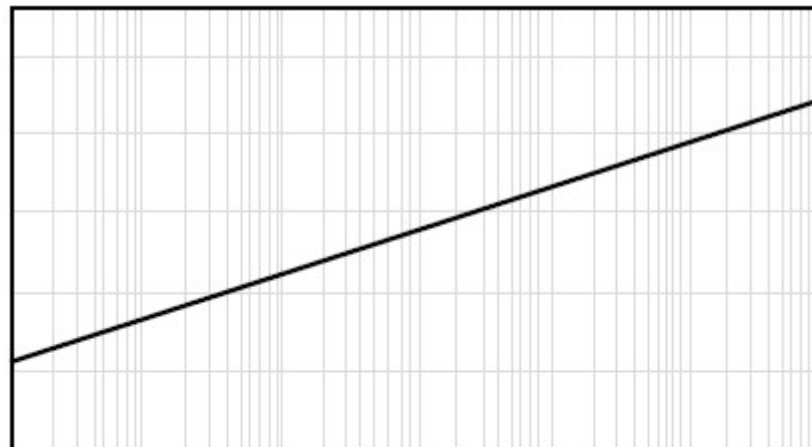
Quartic

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- Linear
- Quadratic
- Cubic
- **Quartic**
- Semi-Log
- Log-Log
- 2-Parameter Exponential
- 4-Parameter Logistic
- 5-Parameter Logistic
- 5-Parameter Logistic Alternate
- Brain Cousens
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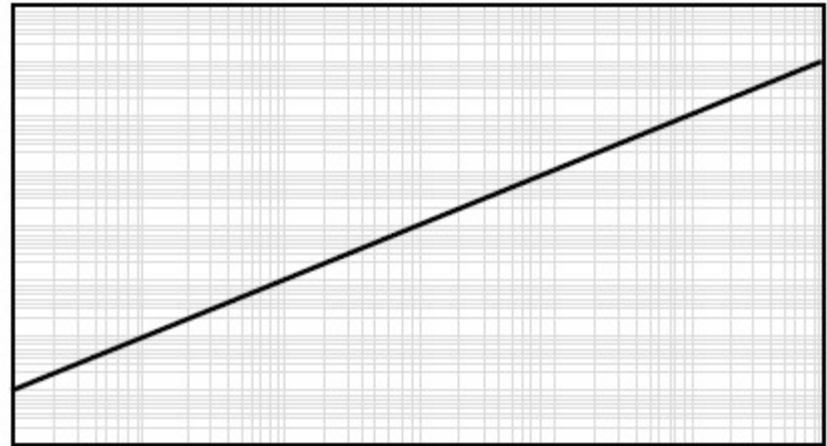
Semi-Log

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- Linear
- Quadratic
- Cubic
- Quartic
- **Semi-Log**
- Log-Log
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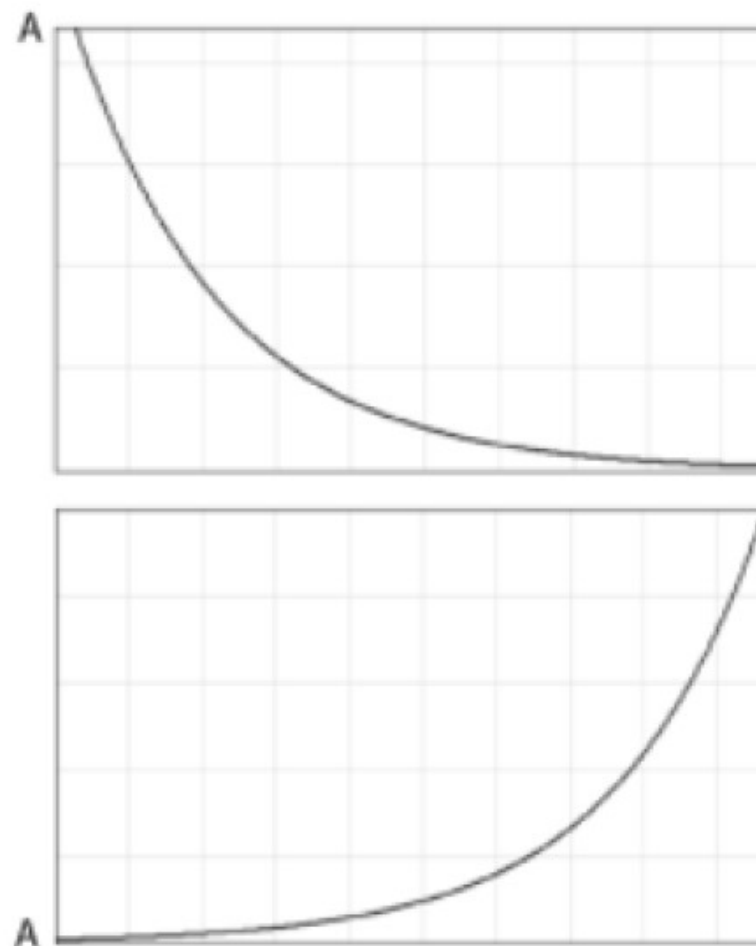
Log-Log

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- Linear
- Quadratic
- Cubic
- Quartic
- Semi-Log
- **Log-Log**
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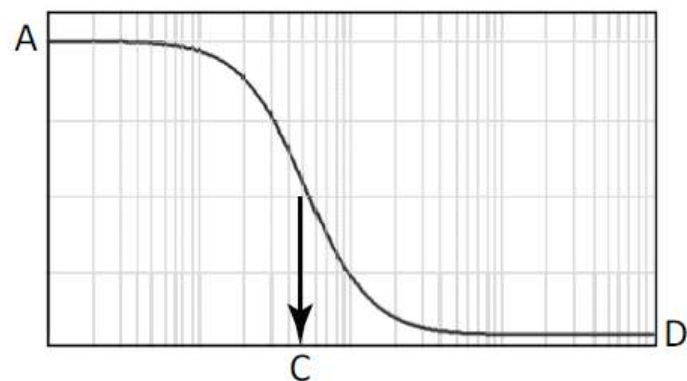
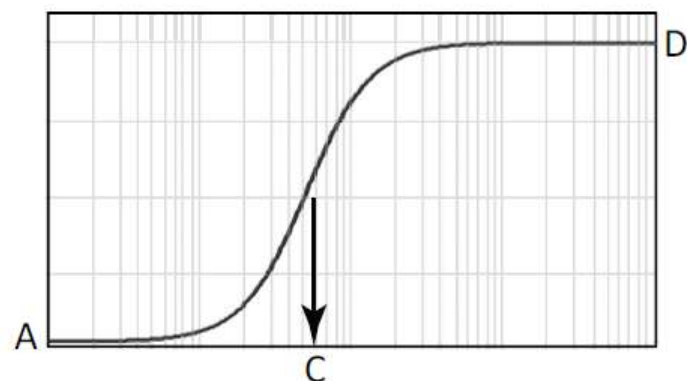
2-Parameter Exponential

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- No Fit
- Linear
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- Quartic
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- Log-Log
- **2-Parameter Exponential**
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4-Parameter Logistic

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- Linear
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- Cubic
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- 2-Parameter Exponential
- **4-Parameter Logistic**
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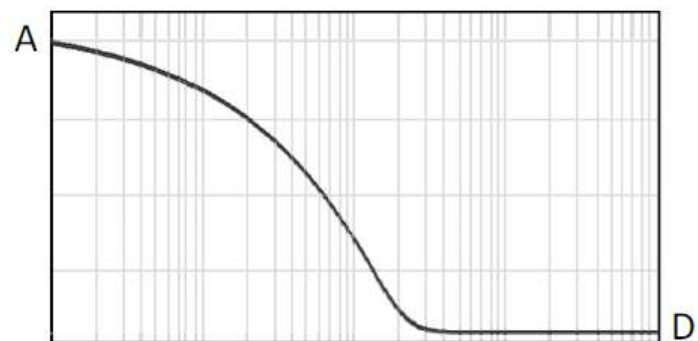
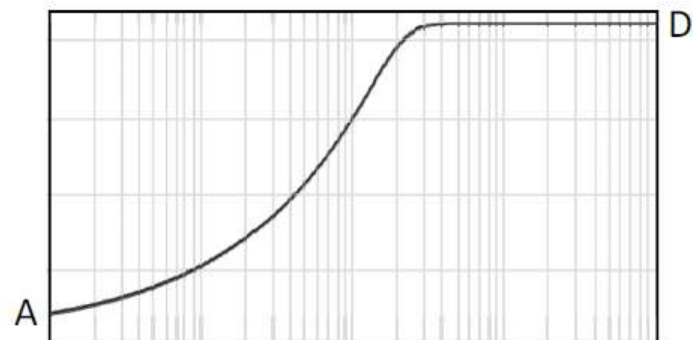


$$y = D + \frac{A - D}{1 + \left(\frac{X}{C}\right)^B}$$



5-Parameter Logistic

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- Quartic
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- **5-Parameter Logistic**
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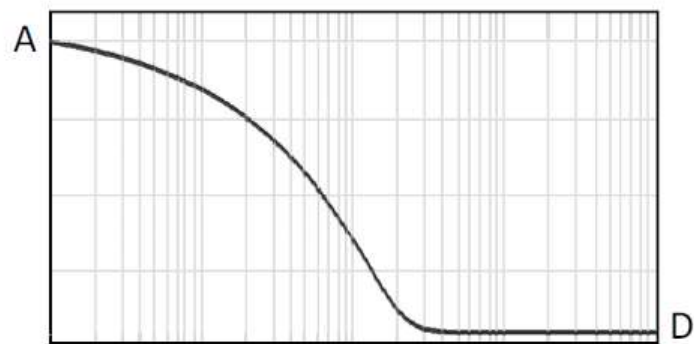
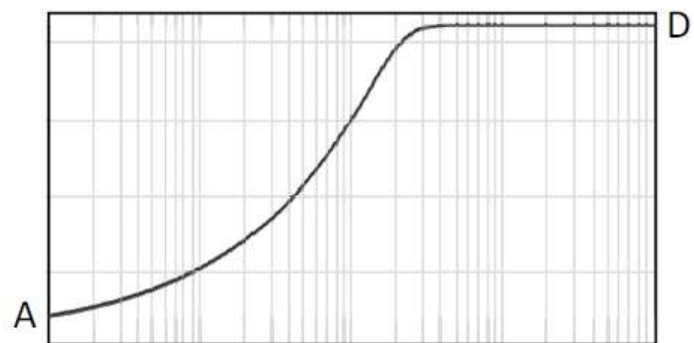


$$y = D + \frac{A - D}{\left\{ 1 + \left(\frac{X}{C} \right)^B \right\}^G}$$



5-Parameter Logistic Alternate

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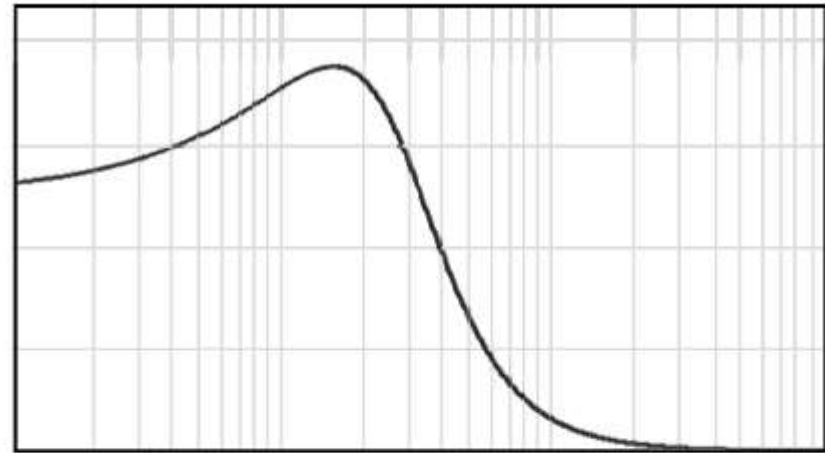


$$y = D + (A - D) \left\{ 1 + \left(2^{\frac{1}{G}} - 1 \right) \left(\frac{X}{C} \right)^{B/2G} \left(1 - 2^{-\frac{1}{C}} \right) \right\}^{-G}$$



Brain Cousens

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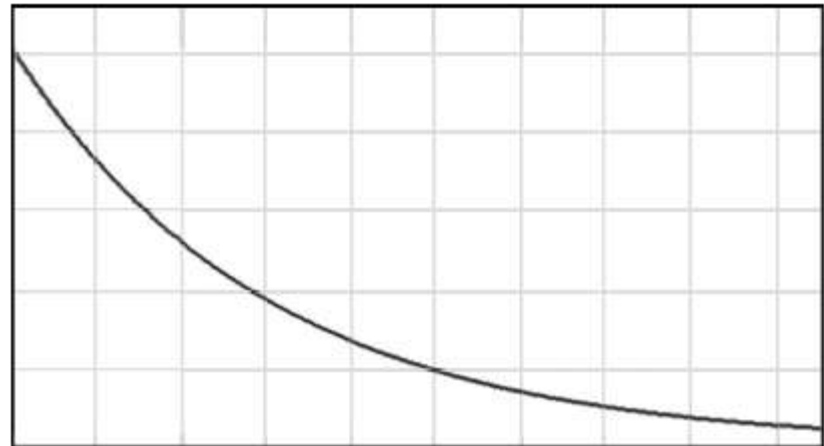
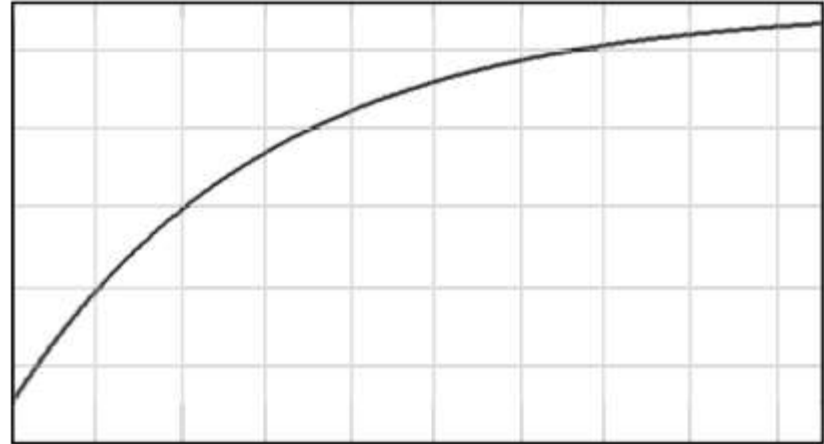


$$y = D + \frac{A - D + Gx}{1 + \left(1 + \frac{2CG}{A - D}\right) \left(\frac{x}{C}\right)^B}$$



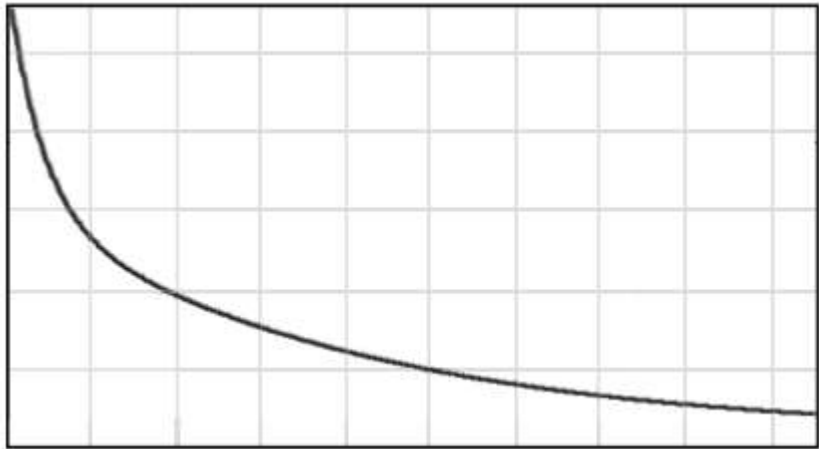
Exponential

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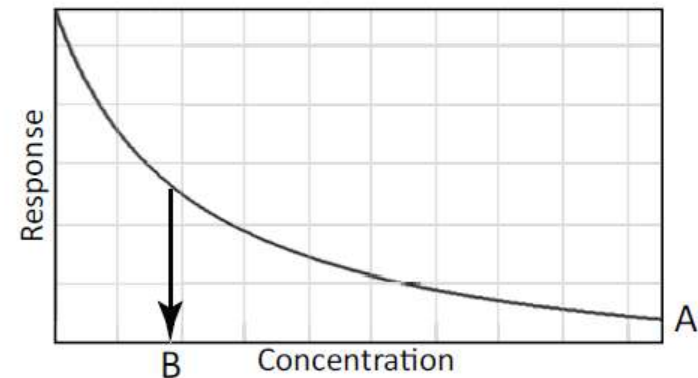
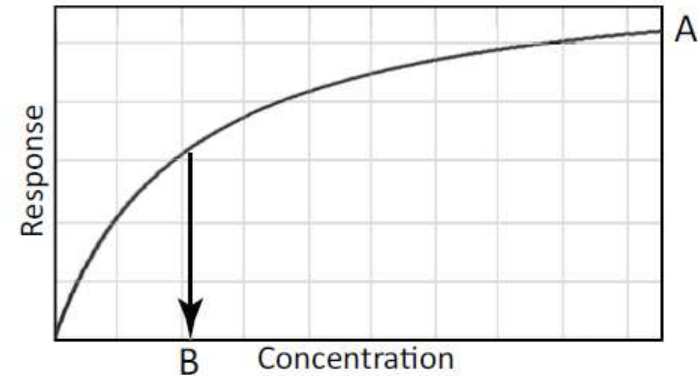
Bi-Exponential

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Rectangular Hyperbola

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- Exponential
- Bi-Exponential
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- Two-Site Competition
- Gaussian

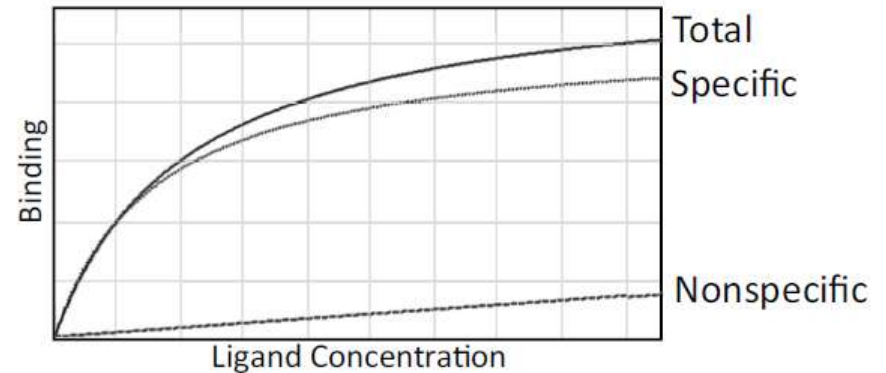


$$y = \frac{Ax}{x + B}$$



RHPlusLinear

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- Linear
- Quadratic
- Cubic
- Quartic
- Semi-Log
- Log-Log
- 2-Parameter Exponential
- 4-Parameter Logistic
- 5-Parameter Logistic
- 5-Parameter Logistic Alternate
- Brain Cousens
- Exponential
- Bi-Exponential
- Rectangular Hyperbola
- **RHPlusLinear**
- Bi-rectangular Hyperbola
- Two-Site Competition
- Gaussian

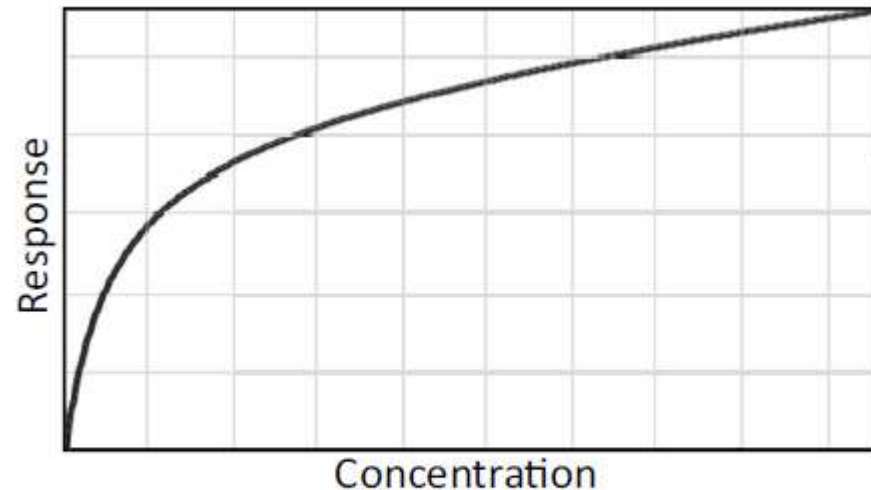


$$y = \frac{Ax}{x + B} + Cx$$



Bi-rectangular Hyperbola

- Press F1 to view details about each curve fit function in the MetaXpress Help file
- No Fit
- Linear
- Quadratic
- Cubic
- Quartic
- Semi-Log
- Log-Log
- 2-Parameter Exponential
- 4-Parameter Logistic
- 5-Parameter Logistic
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- Exponential
- Bi-Exponential
- Rectangular Hyperbola
- RHPlusLinear
- **Bi-rectangular Hyperbola**
- Two-Site Competition
- Gaussian

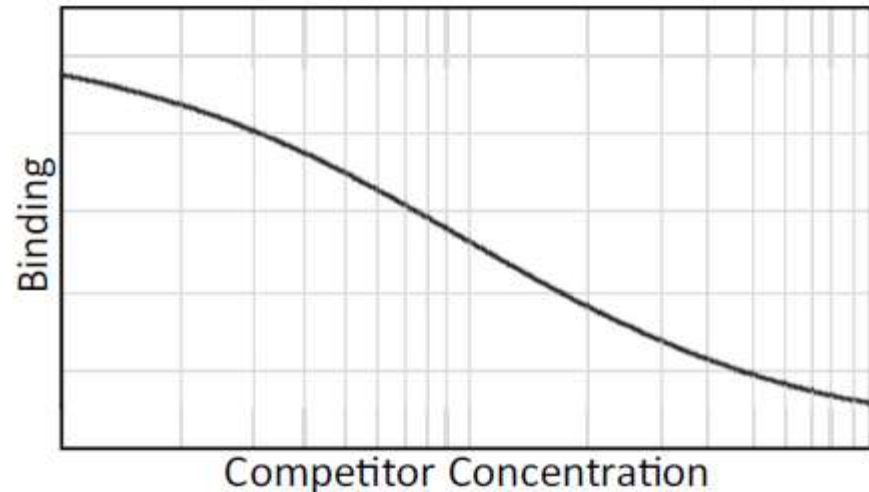


$$y = \frac{Ax}{x + B} + \frac{Cx}{x + D}$$



Two-Site Competition

- Press F1 to view details about each curve fit function in the MetaXpress Help file
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- Linear
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- Cubic
- Quartic
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- RHPlusLinear
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- **Two-Site Competition**
- Gaussian

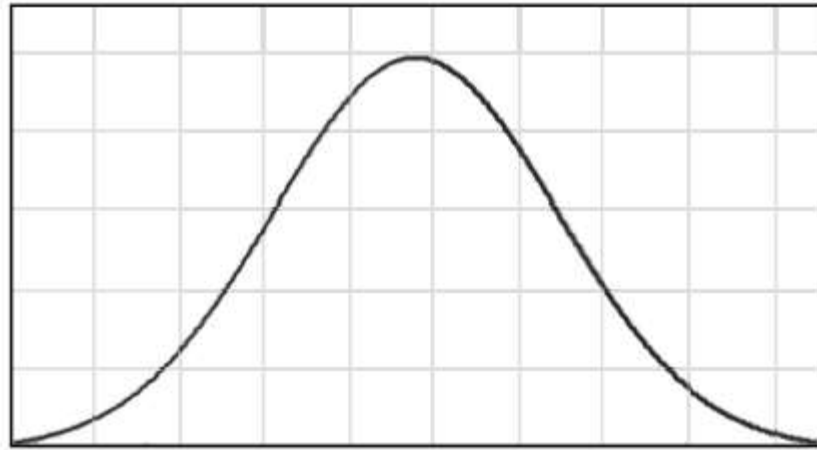


$$y = D + (A - D) \left(\frac{B}{1 + \left(\frac{X}{C}\right)} + \frac{1 - B}{1 + \left(\frac{X}{G}\right)} \right)$$



Gaussian

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- Semi-Log
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- **Gaussian**



$$y = A * e^{-\left(\frac{x - B}{C}\right)^2} + D$$



Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: <http://mdc.custhelp.com/>
- User Forum: <http://metamorph.moleculardevices.com/forum/>
- Request Support: <http://mdc.custhelp.com/app/ask>
- Technical Support can also be reached by telephone:
 - 1 (800) 635-5577
 - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments





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