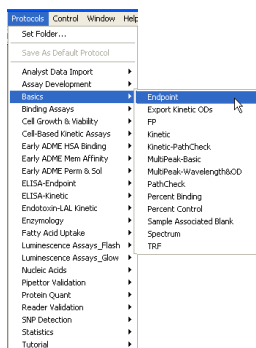


Tips: Always Start with Protocols

- Great starting place for all users
- >120 protocols that can be used directly or customized further
 - ◆ Select from Assays menu
 - ◆ Customize
 - ◆ "Save As..." PROTOCOL file (.ppr)



Tips: Highlighted Protocols

- Basic Endpoint Protocol
 - ◆ Standard curve and interpolated unknowns
- Percent Control
 - ◆ Unknowns are divided by the average of control group
- DNA Plate Blank Method
 - ◆ DNA samples are normalized to 1 cm pathlength & then extinction coefficient is used to calculate concentration
- IC50 Determination
 - ◆ Samples are converted to percent of control, plotted on graph, and then the 50% response value is interpolated from graph
- Michaelis Menten
 - ◆ Standard enzyme kinetic calculations and plots
- All Kinetic ODs for Export
 - ◆ Use when all values during kinetic run are needed

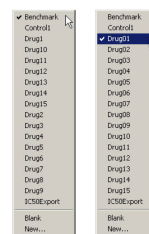
Tips: Template Setup is Critical

- Set-up is critical for ensuring easy Data Analysis
 - ◆ Calculations in Group sections are dependent on template set up
 - ◆ Concentrations, dilutions, and sample descriptors are added in Plate section "template".



Tips: Naming and Sorting

- Sorting
 - ◆ Alphabetic
 - ◆ USE ZEROS for 01-09
 - ◆ Minimize the use of spaces



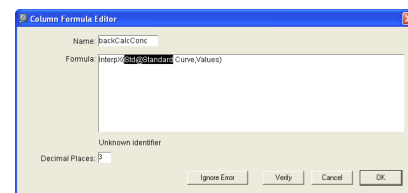
Tips: Naming Conventions in Plate Templates

- Dictates Group section sorting!
 - ◆ Group section names can be customized
 - ◆ Ordering of Sections in a data file/protocol is NOT alphabetical
 - ◆ Order of well names in Group sections IS alphabetical
 - ◆ Well names are read left-to-right

Sample	LotNumber	Well	Value	Mean Value	Std Dev
A01	12355	A1	0.100	0.100	0.007
A02	34543	B1	0.122	0.129	0.008
A03	4354	C1	0.140	0.157	0.011
A04	32453	D1	0.162	0.182	0.014
A05	3405340	E1	0.223	0.234	0.017
A06	34569	F1	0.272	0.286	0.020
A07	54353	G1	0.332	0.346	0.025
A08	73846	H1	0.387	0.427	0.030

Tips: The Effects of Bad Nomenclature

- Group Names are used Everywhere!
- There is a 255 character limit in formulas, so use them wisely
 - ◆ Using special characters or spaces will require the use of single quote (')
 - ◆ 'Standard Curve' vs StandardCurve



Using Formulas & Calculations

- Notes section
 - Only summary formulas are allowed
 - Summaries report single values
- Plate section
 - Used to provide calculations on all data in plate (Reduction)
- Group section
 - Columns formulas are used to provide calculations on all data in table
 - Summary formulas are used to reduce parts of data to a single value
 - Analysis can be carried out in multiple group sections
- Graph section
 - Used to access data from other sections (Plate, Group) directly into a graph

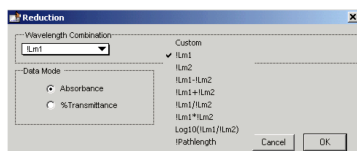
Formulas: Operators, Functions, and Accessors

All Formulas and their parameters are listed in the SoftMax Pro
Formula Reference Guide.

- Accessors
 - Pull or "access" data from different sections
 - Does not normally require data parameters
- Mathematical
 - Combine and Compare numbers, text strings, etc
- Statistical
 - Take a list of numbers as a parameter and return either a single value (summary formulas) or an array of numbers (column formulas)
- Conditional
 - Used to give multiple answers depending on a set of conditions
 - Can return text or numerical results

Using Accessors

- Accessors
 - Use "!" symbol (referred to as "bang") before each accessor
 - Must include "!Well" in front of accessors used in Group sections to identify correct well order from plate
 - Some accessor formulas are available in the Reduction dialog Wavelength Combination pulldown menu.



Samples					
Sample	Wells	Pathlength	OD260	OD280	
TSampleName	WellIDCs	WellPathLength	WellLm1	WellLm2	
S01	A1	1.000	0.100	0.200	
	B1	0.875	0.140	0.254	
	C1	0.750	0.199	0.332	
	D1	0.625	0.292	0.462	
S02	A2	1.000	0.111	0.211	
	B2	0.875	0.154	0.259	
	C2	0.750	0.220	0.351	
	D2	0.625	0.322	0.462	
S03	A3	1.000	0.122	0.222	
	B3	0.875	0.170	0.285	
	C3	0.750	0.243	0.378	
	D3	0.625	0.399	0.516	

Popular Accessors

Accessor	Returns...
!WellLm1	Data from plate that was read at first wavelength
!WellLm2	Data from plate read at second wavelength
!WellValues	Number from plate section using Reduction rules
!WellPathlength	Pathlength measurement for each well
!TimeRun	Times that plate was read during kinetic reads
!WavelengthRun	Wavelengths used during spectral scan
!WellPlateName	Returns the name of the Plate/CuvetteSet section as a text string
!PlateBlank	Average of plate blank
!Factor	Gives second field of info for each well from template
!Concentration or !SampleDescriptor	Returns the numerical value assigned by the user in the Sample Descriptor field.

Simple Mathematical Formulas

- Mathematical Operators
 - + - * / Addition, subtraction, multiplication, division
 - ^ Raises a number to an exponent. Ex: $3^2 = 9$
- Mathematical Functions
 - Abs Gives the absolute value of a number. Ex $\text{Abs}(-10) = 10$
 - Ln Takes the natural logarithm of a number
 - Log10 Takes the logarithm base 10 of a number

Standards1 (µg/ml)						
Sample	Wells	RawOD	StdConc	LogConc	Std%T	AvgStd%T
TSampleName	WellIDCs	WellValues	Concentration	Log10(StdConc)	1000/(10 ^{RawOD(0.719)})	Average(Std%T)
Std01	A8	0.187	45.000	1.65321	54.53	55.06
	B8	0.181			55.60	
	D8	0.179	50.000	1.69897	55.51	67.16
Std02	A9	0.169			57.81	
	B9	0.146	66.250	1.75012	62.28	62.38
	D9	0.145			62.48	
Std04	C10	0.119	62.500	1.79568	67.08	68.54
	D10	0.114			69.09	
	A11	0.087	67.500	1.82930	73.01	73.25
Std05	B11	0.085			73.48	
	C11	0.061	84.375	1.92621	84.76	85.46
	D11	0.046			86.14	

Statistical Formulas

- Many common statistical formulas are readily available as part of a default Group section:
 - Average
 - StDev
 - CV

Samples						
Sample	Wells	Pathlength	OD260	StdDev	StDev#	CV
S01	A1	1.000	0.100	0.083	0.042	46.630
	B1	0.875	0.140			
	C1	0.750	0.199			
	D1	0.625	0.292			
S02	A2	1.000	0.111	0.092	0.046	45.630
	B2	0.875	0.154			
	C2	0.750	0.220			
	D2	0.625	0.322			
S03	A3	1.000	0.122	0.102	0.051	46.630
	B3	0.875	0.170			
	C3	0.750	0.243			
	D3	0.625	0.399			

Conditional Formulas

- Conditionals
 - Can be used to compare answers or eliminate data
 - Comparison operators
 - = Compares if condition is equal
 - > Compares if value is greater than the condition
 - < Compares if value is less than the condition
 - >= Compares if value is greater than or equal to the condition
 - <= Compares if value is less than or equal to the condition
 - <> Compares if value is not equal to the condition
 - Results can be text or numerical, but all results must be the same type
 - If(Values>Minimum,"Pass","Fail") is okay
 - If(Values>Minimum,"Pass", 0) is not

Conditional Formula Structure

- Simple: one condition and two results
 - If(Values>.199,"Pass","Fail")
- Use multiple nested conditionals to handle more possible results.
 - If(Values>.199,"Pass",If(Values<.199,"Fail","Equal"))
- Use multiple conditional operators to determine result
 - If(Values>.199 or Values<.199,"Not Equal","Equal")

Column	Row	Value	Left	Test	Right
501	A1	0.000	Fail	Not Equal	Fail
501	A2	0.140	Fail	Not Equal	Fail
501	A3	0.140	Fail	Not Equal	Fail
501	A4	0.140	Fail	Not Equal	Fail
501	A5	0.140	Fail	Not Equal	Fail
501	A6	0.140	Fail	Not Equal	Fail
501	A7	0.140	Fail	Not Equal	Fail
501	A8	0.140	Fail	Not Equal	Fail
501	A9	0.140	Fail	Not Equal	Fail
501	A10	0.140	Fail	Not Equal	Fail
501	A11	0.140	Fail	Not Equal	Fail
501	A12	0.140	Fail	Not Equal	Fail
501	A13	0.140	Fail	Not Equal	Fail
501	A14	0.140	Fail	Not Equal	Fail
501	A15	0.140	Fail	Not Equal	Fail
501	A16	0.140	Fail	Not Equal	Fail
501	A17	0.140	Fail	Not Equal	Fail
501	A18	0.140	Fail	Not Equal	Fail
501	A19	0.140	Fail	Not Equal	Fail
501	A20	0.140	Fail	Not Equal	Fail

IF Calculation

Name: NewResult1 Hide Name

Description: Total of 2 Log Y values for Left @ Right Rack =

Formula: SumIf (SummingLogY@graphname="Y", LogY@graphname@Y, LogY@graphname@Y)

Decimal Places: 0

Accept Cancel OK

Special Needs in Conditional Formulas

- Conditionals
 - Results in conditional formulas must be all text or all numerical
 - Requires text or numerical "manipulation" of answers
 - Several special formulas exist in SoftMax Pro to help in these situations
 - NAN Examples
 - MakeErr(101) Empty / No number
 - MakeErr(103) Masked
 - MakeErr(115) Pass
 - MakeErr(116) Fail
 - MakeErr(117) High
 - MakeErr(118) Low
- Val(Parameter) Treats text results as a numerical value
- Text(Parameter) Treats numerical answers as a text value
- NoNum Returns an empty "numerical" value

Other Formulas: Getting Information from Graphs

- Extracting values from modeled curve fits

Function	Returns...
AreaUnder(Xvalues,Yvalues)	Trapezoidal area under a curve; default reduction option for kinetic/spectrum
Intercept(Xvalues,Yvalues)	The intercept of a linear regression line defined by y = mx + b
InterpX(PlotName@GraphName,Yvalues)	The X values of the specified plot interpolated using the Y values and curve fit
ParmA(PlotName@GraphName)	The A parameter value of the curve fit assigned to the specified plot
Rsqquared (PlotName@GraphName)	The square of the correlation coefficient of the curve fit assigned to the specified plot
Slope(Xvalues,Yvalues)	The slope of a line that was fit using the linear regression defined by y = mx + b

Other Formulas: Arrays of Numbers

- A number of functions exist to allow access to individual items within lists of numbers (e.g. column in group table), to eliminate portions of lists from calculations, and even manipulate text.
 - Particularly useful when dealing with arrays of data obtained from kinetic and spectrum reads

Function	Returns...
FirstItem(Parameter)	The first item in a list or array of numbers (e.g. the first OD in a kinetic run)
ItemCount	The count, or number of items, in a list or array of numbers
NthItem(Parameter1,N)	The value of the Nth item in Parameter1, where Parameter1 is a list or array of numbers, and N is a number.
NullOutside(Parameter1,Parameter2,Parameter3)	Omits selected items in a list or array of numbers defined by Parameter1. Parameter2 is a number indicating at which item to stop omitting, and Parameter 3 is a number indicating at which item to resume omitting.

Weighting

- What is it?
 - Weighting is used to overcome range errors at low and high concentrations
 - A commonly used weighting scheme is 1/variance, allowing more "weight" to be assigned to data points with low variance.
 - Variance is calculated as $SDEV^2$
 - Custom weighting available in SoftMax Pro v5
 - Weighting is ONLY available for the 4 and 5-parameter curve fits
- Best used with:
 - Existing/historical standards data
 - Comparing historical & newly read standards to detect out-of-spec results

PLA - Weighted

GraphView 4-Parameter

Sample file:
C:\Program Files\Molecular Devices\
SoftMax Pro v5.2\Tutorial\
Statistics sample data

4- And 5 Parameter Settings

Curve Fit Weighting

☒ Fixed Weight for all points

☐ Variable Weight for each point

Manually Set Parameters

☐ A

☐ B

☐ C

☐ D

☐ E

Parallel Line Analysis

☐ Use parallel line analysis

Standard Curve:

Cancel OK

Questions?

- Presentation, Data Files, and Solutions will be available shortly
- Specific formula writing questions:
Support@moldev.com