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Moving medicine forward.

Outlier Analysis for Relative Potency Assays Using SoftMax Pro Function for Rosner Extreme Studentized Deviate Test

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ABZENA

Who we are

Abzena is the leading, end-to-end bioconjugate and complex biologics CDMO+CRO, focused on rapidly moving medicines forward to patients in need.

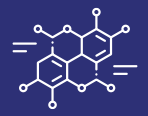
How we support our customers

Capabilities spanning from discovery through commercial launch

Experts in developing:

> mABs | Fusion Proteins | ADCs | Bioconjugates | Biologics | Oligonucleotide Conjugates | Vaccines | Radioconjugates

Fully integrated or tailored services to meet customers needs:



Discovery, Design and Lead Selection

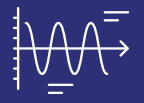
Bioassays and Immunogenicity

Protein Engineering and Developability

Mammalian Cell Line Development

Chemistry and Bioconjugation

Formulation Development



Analytical and Process Development

CTM and Commercial GMP Manufacturing

Outlier Analysis using SoftMax Pro software

Our outlier analysis approach for dose-response curves in relative potency assays utilizes customized SoftMax Pro[1] data acquisition and analysis software templates to perform all calculations, processing and reporting without any data transfer to another statistical program.

Outliers are determined based on the residuals from independent curve fit using SoftMax Pro function for Rosner extreme studentized deviate test: “ESD Mark Outliers (data, outliers, significance)”.

Distributions of the residuals are tested for normality with and without identified outliers using SoftMax Pro function for Shapiro-Wilk Royston probability test: “ShapiroWilkRoystonProbability(data)”. The rationality of outlier exclusion is based on the comparisons of the results of normality tests using distribution of the residuals of the standard curve without outliers typical for relative potency assay.

[1]SoftMax Pro software developed by Molecular Devices

Example of SoftMax Pro template

Reference, Samples and Control tested as 8-point (7 non-zero) dose-response curves (DRC), in triplicates, 3-fold serial dilutions using 96-well plate format

Four-parameter logistic (4-PL) regression model was used for dose-response curve fitting:

- Independent – for Outlier analysis and assessment of parallelism with Reference curve
- Global – for paired constrained fits of each Sample and Control with Reference

Negative Control (NC) 0-concentration, used to fix lower asymptotes for all curves

ESD Mark Outliers Settings: Max Outliers per curve = 5, Significance = 0.05

	1	2	3	4	5	6	7	8	9	10	11	12
A	Refere...	Sampl...	Sample	Control	Refere...	Sampl...	Sample	Control	Refere...	Sampl...	Sample	Control
B	1.667	1.667	1.667	1.667	1.667	1.667	1.667	1.667	1.667	1.667	1.667	1.667
C	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556	0.556
D	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185
E	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062
F	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021
G	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

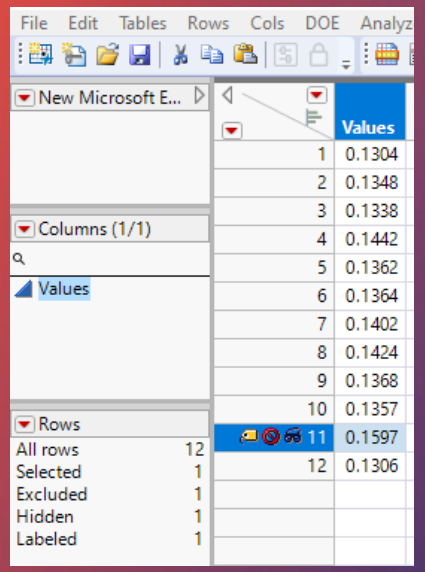
NC

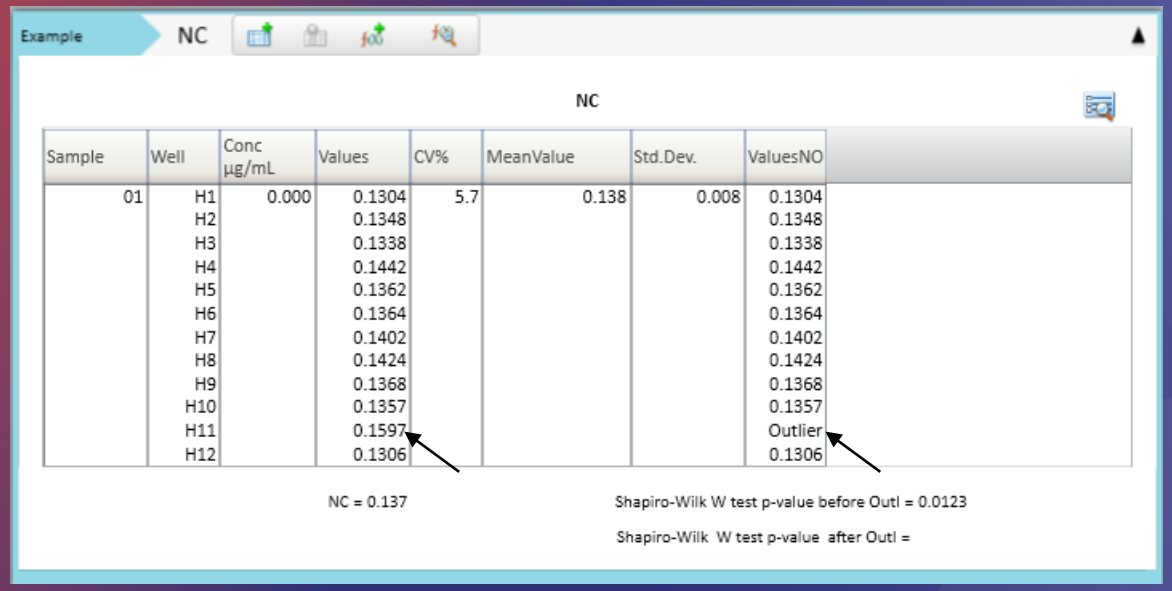
Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.213	2.098	2.335	2.286	2.283	2.287	2.277	2.286	2.311	2.353	2.300	2.368
B	2.120	2.080	2.210	2.182	2.175	2.225	2.222	2.123	2.236	2.207	2.206	2.246
C	1.810	1.851	1.889	1.985	1.900	2.001	1.889	1.903	1.964	1.954	1.938	1.983
D	0.862	0.944	0.960	0.941	0.961	0.975	0.775	0.954	0.958	0.985	0.942	0.959
E	0.277	0.288	0.301	0.285	0.315	0.316	0.318	0.288	0.302	0.296	0.294	0.281
F	0.153	0.163	0.155	0.167	0.131	0.173	0.175	0.162	0.161	0.157	0.162	0.162
G	0.136	0.149	0.139	0.140	0.146	0.145	0.143	0.140	0.135	0.144	0.141	0.139
H	0.130	0.135	0.134	0.144	0.136	0.136	0.140	0.142	0.137	0.136	0.160	0.131

Raw Data

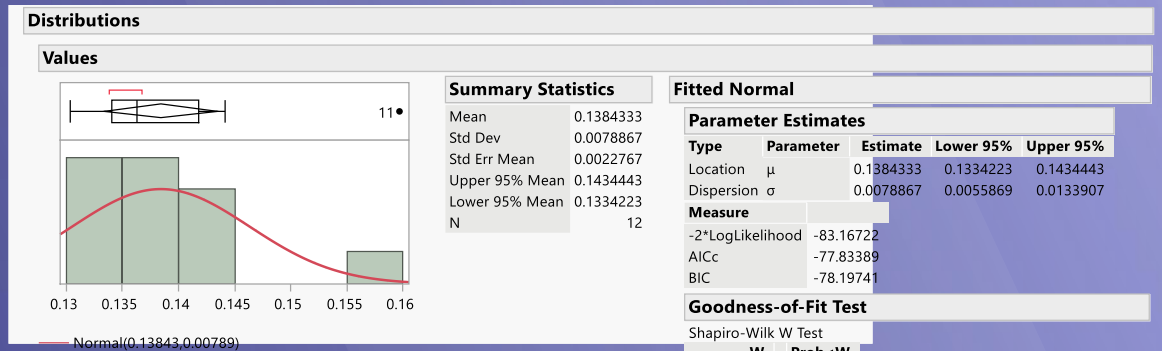
Negative Control - One Outlier Detected

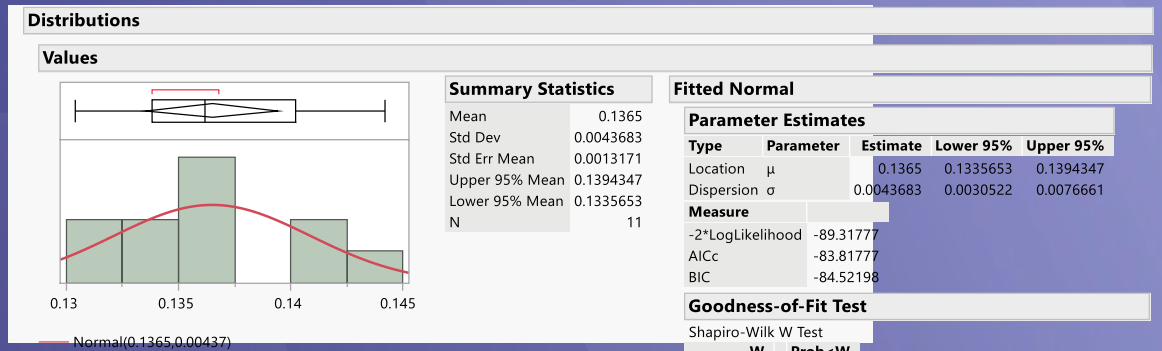




SoftMax Pro: ESDMarkOutliers(Values, Max # of Outliers , Significance)

SoftMax Pro "ShapiroWilkRoystonProbability(data)" function requires no less than 12 data points





JMP: Distribution, Outlier Box Plot

Reference DRC – no Outliers detected

Example | Reference

Reference

Sample	Conc µg/mL	Wells	Values	CV%	FitValue	ValueResiduals	ValueResidOutl	ValuewoOutl
01	5.000	A1	2.213	2.2	2.252	-0.039	-0.039	2.213
		A5	2.283			0.031	0.031	2.283
		A9	2.311			0.059	0.059	2.311
02	1.667	B1	2.120	2.6	2.202	-0.081	-0.081	2.120
		B5	2.175			-0.026	-0.026	2.175
		B9	2.236			0.034	0.034	2.236
03	0.556	C1	1.810	4.1	1.881	-0.071	-0.071	1.810
		C5	1.900			0.019	0.019	1.900
		C9	1.964			0.082	0.082	1.964
04	0.185	D1	0.862	6.1	0.933	-0.070	-0.070	0.862
		D5	0.961			0.029	0.029	0.961
		D9	0.958			0.025	0.025	0.958
05	0.062	E1	0.277	6.6	0.290	-0.013	-0.013	0.277
		E5	0.315			0.025	0.025	0.315
		E9	0.302			0.012	0.012	0.302
06	0.021	F1	0.153	10.2	0.158	-0.005	-0.005	0.153
		F5	0.131			-0.026	-0.026	0.131
		F9	0.161			0.003	0.003	0.161
07	0.007	G1	0.136	4.4	0.139	-0.004	-0.004	0.136
		G5	0.146			0.007	0.007	0.146
		G9	0.135			-0.004	-0.004	0.135

Noutl = 0

D parameters:

DReference = 2.26

vDReference = 0.23

B parameters:

Breference = 1.857

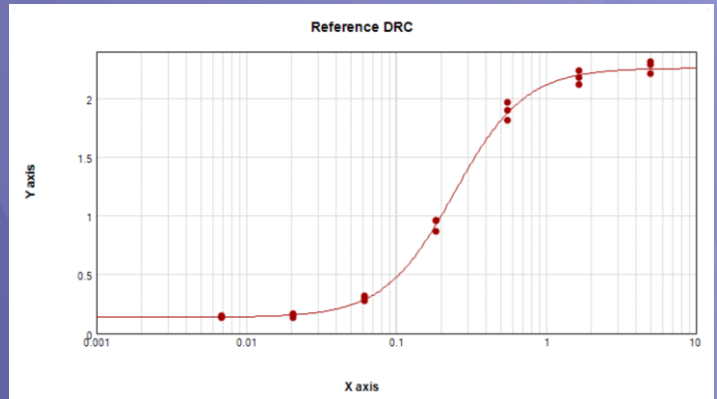
vBReference = 3.455

Outl = -0.039
0.031
0.059
-0.081
-0.026
0.034
-0.071
0.019
0.082
-0.070
0.029
0.025
-0.013
0.025
0.012
-0.005
-0.026
0.003
-0.004
0.007
-0.004

MCV = 2.4

Shapiro-Wilk W test p-value before Outl = 0.6288

Shapiro-Wilk W test p-value after Outl = 0.6288



Plot1 (Reference: ValuewoOutl vs Conc) Weighting: Fixed

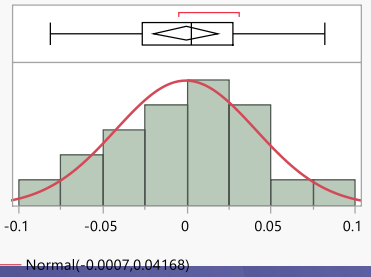
Curve Fit Results ▲

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

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot1	A	0.137	Fixed	[0.137, 0.137]
R^2 = 0.998	B	1.857	0.082	[1.685, 2.028]
EC50 = 0.244	C	0.244	0.007	[0.229, 0.258]
	D	2.260	0.021	[2.215, 2.304]

Distributions

ValueResiduals



Summary Statistics

Mean	-0.000706
Std Dev	0.0416812
Std Err Mean	0.0090956
Upper 95% Mean	0.018267
Lower 95% Mean	-0.019679
N	21

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	µ	-0.000706	-0.019679	0.018267
Dispersion	σ	0.0416812	0.0318886	0.0601906

Measure	
-2*LogLikelihood	-74.86819
AICc	-70.20152
BIC	-68.77915

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.965313	0.6288

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

SoftMax Pro: ESDMarkOutliers(ValueResiduals, Max # of Outliers , Significance)

JMP: Distribution, Outlier Box Plot

Sample DRC – One Outlier Detected

Example | Sample

Sample

Sample	Conc µg/mL	Wells	Values	CV%	FitValue	ValueResiduals	ValueResidOutl	ValuewoOutl
01	5.000	A3	2.335	1.3	2.292	0.044	0.044	2.335
		A7	2.277			-0.015	-0.015	2.277
		A11	2.300			0.008	0.008	2.300
02	1.667	B3	2.210	0.4	2.236	-0.026	-0.026	2.210
		B7	2.222			-0.014	-0.014	2.222
		B11	2.206			-0.030	-0.030	2.206
03	0.556	C3	1.889	1.5	1.888	0.001	0.001	1.889
		C7	1.889			0.002	0.002	1.889
		C11	1.938			0.050	0.050	1.938
04	0.185	D3	0.960	11.4	0.907	0.052	0.052	0.960
		D7	0.775			-0.133	Outlier	
		D11	0.942			0.034	0.034	0.942
05	0.062	E3	0.301	4.0	0.282	0.018	0.018	0.301
		E7	0.318			0.036	0.036	0.318
		E11	0.294			0.012	0.012	0.294
06	0.021	F3	0.155	6.0	0.157	-0.001	-0.001	0.155
		F7	0.175			0.018	0.018	0.175
		F11	0.162			0.006	0.006	0.162
07	0.007	G3	0.139	1.3	0.139	0.000	0.000	0.139
		G7	0.143			0.004	0.004	0.143
		G11	0.141			0.002	0.002	0.141

ChiSqdTotal = 0 dfTotal = 35 alpha = 0.10 Noutl = 1

SigmaSqd = 0 t = 1.690

D parameters:

DSample2 = 2.299

rD = 0.983

vDSample2 = 0.241

gD = 0.000

hwD = 0.017

rDCIUpper = 1.000

rDCILower = 0.966

B parameters:

BSample2 = 1.801

rB = 1.031

vBSample2 = 3.145

gB = 0.003

hwB = 0.084

rBCIUpper = 1.118

rBCILower = 0.951

Outl = 0.044, -0.015, 0.008, -0.026, -0.014, -0.030, 0.001, 0.002, 0.050, 0.052, Outlier, 0.034, 0.018, 0.036, 0.012, -0.001, 0.018, 0.006, 0.000, 0.004, 0.002

MCV = 6.0

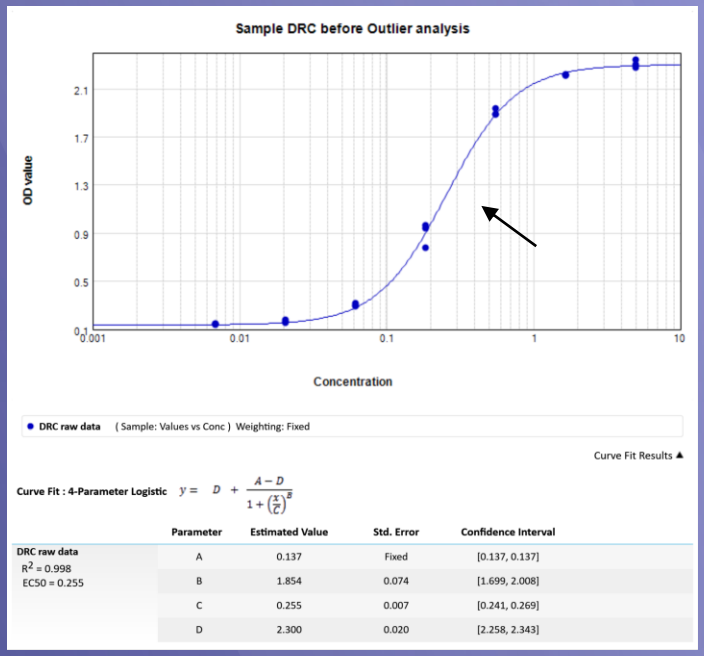
Shapiro-Wilk W test p-value before Outl = 0.0006

Shapiro-Wilk W test p-value after Outl = 0.3815

Chi-Squared ptobability = 0.998

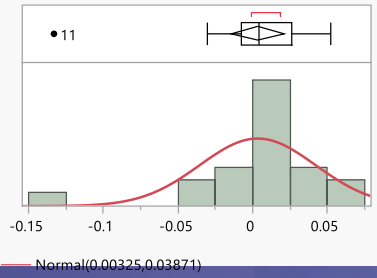
F-test probability = 0.256

HasData = TRUE



Distributions

ValueResiduals



Summary Statistics

Mean	0.0032518
Std Dev	0.0387096
Std Err Mean	0.0084471
Upper 95% Mean	0.0208722
Lower 95% Mean	-0.014369
N	21

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	µ	0.0032518	-0.014369	0.0208722
Dispersion	σ	0.0387096	0.0296151	0.0558993

Measure	
-2*LogLikelihood	-77.97463
AICc	-73.30796
BIC	-71.88558

Goodness-of-Fit Test

Shapiro-Wilk W Test

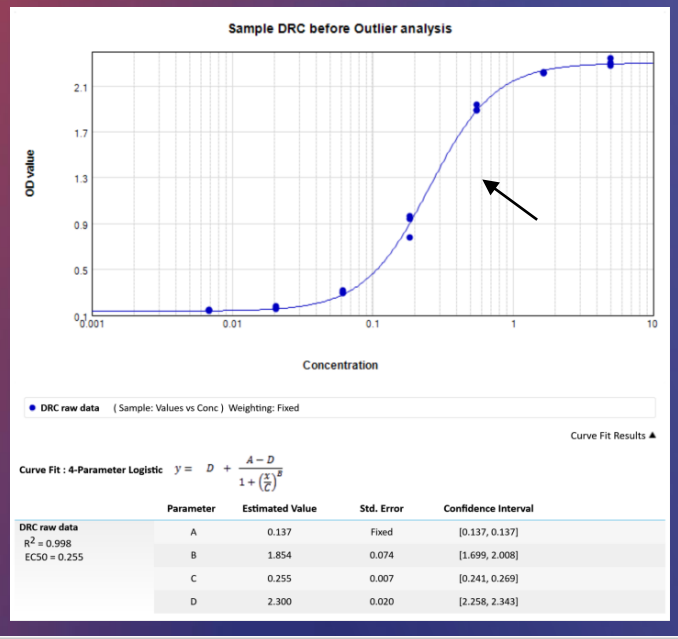
W	Prob<W
0.799183	0.0006 *

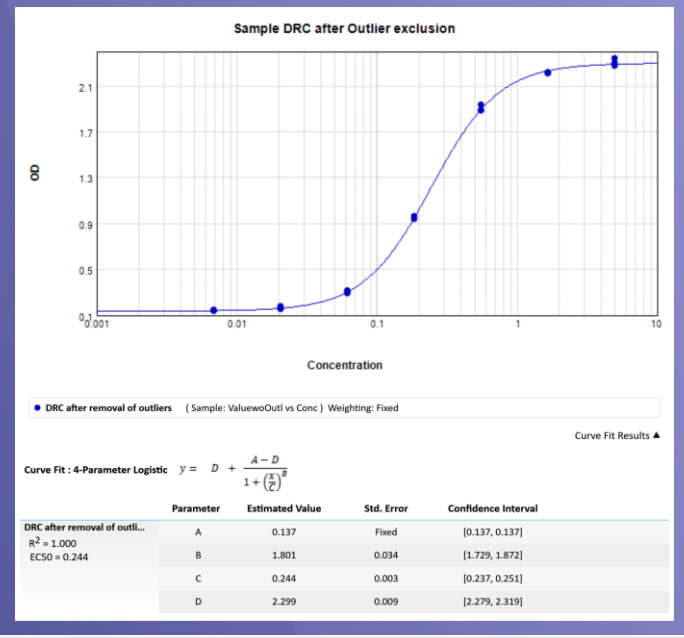
Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

SoftMax Pro: ESDMarkOutliers(ValueResiduals, Max # of Outliers , Significance)

JMP: Distribution, Outlier Box Plot

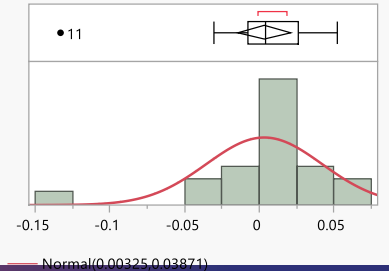
Sample DRC Independent Fits Before & After Outlier Exclusion





Distributions

ValueResiduals



Summary Statistics	
Mean	0.0032518
Std Dev	0.0387096
Std Err Mean	0.0084471
Upper 95% Mean	0.0208722
Lower 95% Mean	-0.014369
N	21

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.0032518	-0.014369	0.0208722
Dispersion	σ	0.0387096	0.0296151	0.0558993

Measure	
-2*LogLikelihood	-77.97463
AICc	-73.30796
BIC	-71.88558

Goodness-of-Fit Test

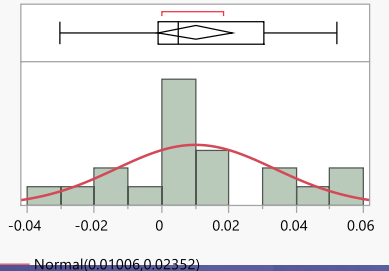
Shapiro-Wilk W Test

W	Prob<W
0.799183	0.0006 *

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Distributions

ValueResiduals



Summary Statistics	
Mean	0.0100588
Std Dev	0.0235173
Std Err Mean	0.0052586
Upper 95% Mean	0.0210652
Lower 95% Mean	-0.000948
N	20

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.0100588	-0.000948	0.0210652
Dispersion	σ	0.0235173	0.0178847	0.0343487

Measure	
-2*LogLikelihood	-94.24325
AICc	-89.53737
BIC	-88.25179

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.950932	0.3815

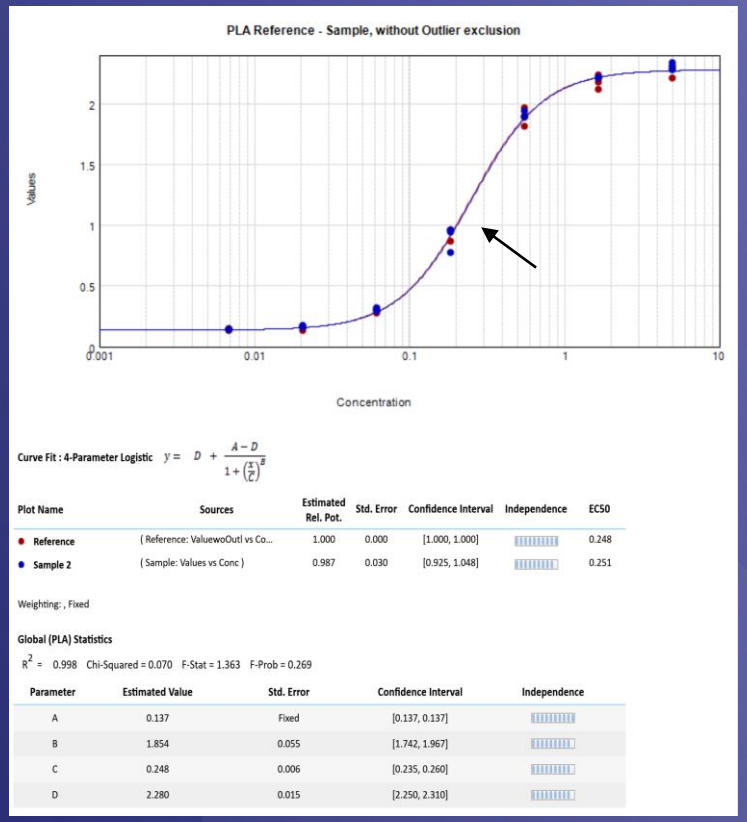
Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Sample Relative Potency Estimates Before & After Exclusion of Outliers

Calculation of Relative Potency and RP Confidence Intervals based the ratios of EC50 (Parameter C) from Paired Global 4PL fits after Parallelism testing

Test for parallelism based on SoftMax Pro Fieller's Theorem protocol for Upper asymptotes (Parameter D) and Slopes (Parameter B) ratios from Independent 4PL Fits (the output of Fieller's theorem is the confidence interval for the ratio).

The suitability criteria for parallelism: the lower confidence limit must be > 0.8 and the upper confidence limit must be < 1.25, for both ratios.



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

Plot Name	Sources	Estimated Rel. Pot.	Std. Error	Confidence Interval	Independence	EC50
Reference	(Reference: ValuewoOutl vs Co...	1.000	0.000	[1.000, 1.000]		0.248
Sample 2	(Sample: Values vs Conc)	0.987	0.030	[0.925, 1.048]		0.251

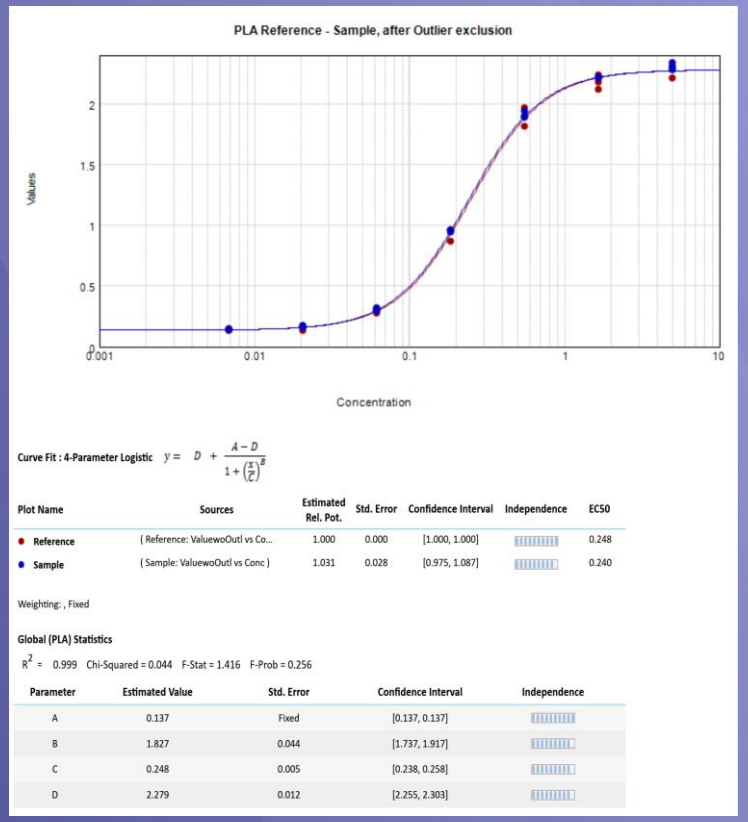
Weighting: , Fixed

Global (PLA) Statistics

R^2 = 0.998 Chi-Squared = 0.070 F-Stat = 1.363 F-Prob = 0.269

Parameter	Estimated Value	Std. Error	Confidence Interval	Independence
A	0.137	Fixed	[0.137, 0.137]	
B	1.854	0.055	[1.742, 1.967]	
C	0.248	0.006	[0.235, 0.260]	
D	2.280	0.015	[2.250, 2.310]	

Before: Relative Potency = 0.987 (98.7%)



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

Plot Name	Sources	Estimated Rel. Pot.	Std. Error	Confidence Interval	Independence	EC50
Reference	(Reference: ValuewoOutl vs Co...	1.000	0.000	[1.000, 1.000]		0.248
Sample	(Sample: ValuewoOutl vs Conc)	1.031	0.028	[0.975, 1.087]		0.240

Weighting: , Fixed

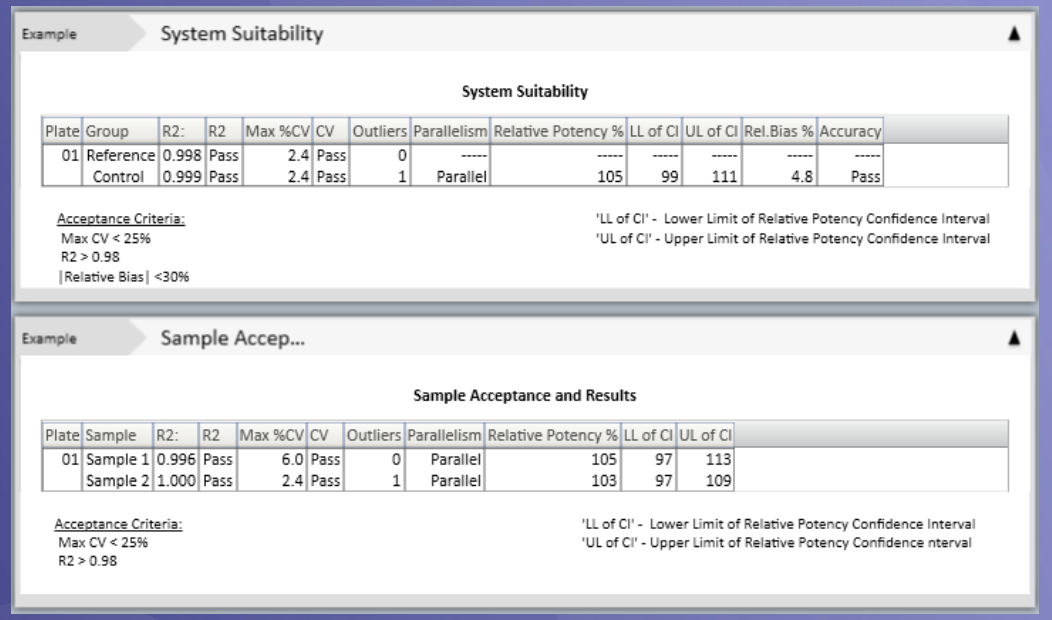
Global (PLA) Statistics

R^2 = 0.999 Chi-Squared = 0.044 F-Stat = 1.416 F-Prob = 0.256

Parameter	Estimated Value	Std. Error	Confidence Interval	Independence
A	0.137	Fixed	[0.137, 0.137]	
B	1.827	0.044	[1.737, 1.917]	
C	0.248	0.005	[0.238, 0.258]	
D	2.279	0.012	[2.255, 2.303]	

After: Relative Potency = 1.031 (103.1%)

QUESTIONS?



Example — System Suitability

System Suitability

Plate	Group	R2:	R2	Max %CV	CV	Outliers	Parallelism	Relative Potency %	LL of CI	UL of CI	Rel.Bias %	Accuracy
01	Reference	0.998	Pass	2.4	Pass	0	-----	-----	-----	-----	-----	-----
	Control	0.999	Pass	2.4	Pass	1	Parallel	105	99	111	4.8	Pass

Acceptance Criteria:
Max CV < 25%
R2 > 0.98
|Relative Bias| <30%

'LL of CI' - Lower Limit of Relative Potency Confidence Interval
'UL of CI' - Upper Limit of Relative Potency Confidence Interval

Example — Sample Accep...

Sample Acceptance and Results

Plate	Sample	R2:	R2	Max %CV	CV	Outliers	Parallelism	Relative Potency %	LL of CI	UL of CI
01	Sample 1	0.996	Pass	6.0	Pass	0	Parallel	105	97	113
	Sample 2	1.000	Pass	2.4	Pass	1	Parallel	103	97	109

Acceptance Criteria:
Max CV < 25%
R2 > 0.98

'LL of CI' - Lower Limit of Relative Potency Confidence Interval
'UL of CI' - Upper Limit of Relative Potency Confidence nterval

ABZENA

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