

TECHNOLOGY PREVIEW 03/2020 – This report has been generated with the upcoming Dose-Response-Analysis Package for PLA 3.0.5 (or higher). The package has not been released and has not been finally verified for production use. Contact Stegmann Systems (<http://support.bioassay.de>) to report issues or suggest additional features.

4 PLF - Curve Comparison

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Calculation information

Calculation performed:	24.03.2020 12:13:05, odin.kleuser@stegmannsystems.com (PLA 3.0.4 Build 762, nbersatz02, 10014)
Messages (warning):	[W5228] The fit of sample CURVE 1 has a reduced accuracy. [W5228] The fit of sample CURVE 2 has a reduced accuracy. [W5228] The fit of sample CURVE 3 has a reduced accuracy.
Report generated:	25.03.2020 08:01:46, Administrator (PLA 3.0.4 Build 762, NBAFR01, 10014)

Signatures

Responsibility	Review	Approval
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Documentation

Date	30.07.2019 07:49:21
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DOCUMENT-26



741AC16E-3F79-46E6-A8A7-5CB4AD388293

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Assay overview

Assay elements	Summary
<ul style="list-style-type: none"> ■ CURVE 1 ■ CURVE 2 ■ CURVE 3 	Reference standard, multi-dose (8 dilution steps, 3 replicates) Test sample, multi-dose (8 dilution steps, 3 replicates) Test sample, multi-dose (8 dilution steps, 3 replicates)
Property	Value
Response data processing	Response data is proceed as it is.
Analytical model	
Model	4 parameter logistic fit
Dose transformation	Logarithmic: binary logarithm (base 2)

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A		[1] 211.914	[1] 201.570	[1] 197.065	[1] 250.195	[1] 250.704	[1] 258.680	[1] 292.566	[1] 295.986	[1] 307.285		
B		[2] 199.793	[2] 195.209	[2] 199.131	[2] 247.798	[2] 232.954	[2] 248.163	[2] 304.102	[2] 282.559	[2] 293.543		
C		[3] 177.245	[3] 172.293	[3] 175.076	[3] 216.722	[3] 210.835	[3] 225.390	[3] 307.150	[3] 296.291	[3] 295.617		
D		[4] 103.475	[4] 104.818	[4] 103.207	[4] 125.051	[4] 126.280	[4] 137.138	[4] 283.193	[4] 286.593	[4] 270.463		
E		[5] 17.180	[5] 26.286	[5] 33.826	[5] 28.801	[5] 39.187	[5] 34.210	[5] 133.847	[5] 144.474	[5] 150.100		
F		[6] 2.530	[6] 12.831	[6] 24.599	[6] 16.370	[6] 19.975	[6] 10.733	[6] 19.208	[6] 24.554	[6] 21.673		
G		[7] 16.879	[7] 1.757	[7] 18.004	[7] 14.309	[7] 20.651	[7] 15.228	[7] 17.298	[7] 6.868	[7] 10.905		
H		[8] 23.649	[8] 1.288	[8] 8.341	[8] 6.013	[8] 14.718	[8] 19.978	[8] 11.518	[8] 14.259	[8] 6.937		

Dataset

	Assay Element	Group ID	Dilution step	Response	Technical Outlier	Row
1	■ CURVE 1	1	1	211.91351	false	1
2	■ CURVE 1	1	1	201.57012	false	1
3	■ CURVE 1	1	1	197.06491	false	1
4	■ CURVE 1	1	2	199.79343	false	2
5	■ CURVE 1	1	2	195.20899	false	2
6	■ CURVE 1	1	2	199.13072	false	2
7	■ CURVE 1	1	3	177.24509	false	3
8	■ CURVE 1	1	3	172.29252	false	3
9	■ CURVE 1	1	3	175.07573	false	3
10	■ CURVE 1	1	4	103.47476	false	4
11	■ CURVE 1	1	4	104.81782	false	4
12	■ CURVE 1	1	4	103.20709	false	4
13	■ CURVE 1	1	5	17.17979	false	5
14	■ CURVE 1	1	5	26.28608	false	5
15	■ CURVE 1	1	5	33.82634	false	5
16	■ CURVE 1	1	6	2.53044	false	6
17	■ CURVE 1	1	6	12.83126	false	6
18	■ CURVE 1	1	6	24.59858	false	6
19	■ CURVE 1	1	7	16.87861	false	7
20	■ CURVE 1	1	7	1.75747	false	7
21	■ CURVE 1	1	7	18.00377	false	7
22	■ CURVE 1	1	8	23.64852	false	8
23	■ CURVE 1	1	8	1.28774	false	8
24	■ CURVE 1	1	8	8.34130	false	8
25	■ CURVE 3	3	1	250.19531	false	1
26	■ CURVE 3	3	1	250.70353	false	1
27	■ CURVE 3	3	1	258.68016	false	1
28	■ CURVE 3	3	2	247.79815	false	2
29	■ CURVE 3	3	2	232.95436	false	2
30	■ CURVE 3	3	2	248.16306	false	2
31	■ CURVE 3	3	3	216.72223	false	3

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	Assay Element	Group ID	Dilution step	Response	Technical Outlier	Row
32	CURVE 3	3	3	210.83509	false	3
33	CURVE 3	3	3	225.38977	false	3
34	CURVE 3	3	4	125.05083	false	4
35	CURVE 3	3	4	126.28014	false	4
36	CURVE 3	3	4	137.13765	false	4
37	CURVE 3	3	5	28.80068	false	5
38	CURVE 3	3	5	39.18680	false	5
39	CURVE 3	3	5	34.20951	false	5
40	CURVE 3	3	6	16.37005	false	6
41	CURVE 3	3	6	19.97453	false	6
42	CURVE 3	3	6	10.73337	false	6
43	CURVE 3	3	7	14.30931	false	7
44	CURVE 3	3	7	20.65082	false	7
45	CURVE 3	3	7	15.22821	false	7
46	CURVE 3	3	8	6.01348	false	8
47	CURVE 3	3	8	14.71754	false	8
48	CURVE 3	3	8	19.97839	false	8
49	CURVE 2	2	1	292.56633	false	1
50	CURVE 2	2	1	295.98557	false	1
51	CURVE 2	2	1	307.28481	false	1
52	CURVE 2	2	2	304.10191	false	2
53	CURVE 2	2	2	282.55916	false	2
54	CURVE 2	2	2	293.54266	false	2
55	CURVE 2	2	3	307.14955	false	3
56	CURVE 2	2	3	296.29104	false	3
57	CURVE 2	2	3	295.61676	false	3
58	CURVE 2	2	4	283.19310	false	4
59	CURVE 2	2	4	286.59337	false	4
60	CURVE 2	2	4	270.46342	false	4
61	CURVE 2	2	5	133.84735	false	5
62	CURVE 2	2	5	144.47419	false	5
63	CURVE 2	2	5	150.09980	false	5
64	CURVE 2	2	6	19.20772	false	6
65	CURVE 2	2	6	24.55355	false	6
66	CURVE 2	2	6	21.67286	false	6
67	CURVE 2	2	7	17.29848	false	7
68	CURVE 2	2	7	6.86793	false	7
69	CURVE 2	2	7	10.90535	false	7
70	CURVE 2	2	8	11.51796	false	8
71	CURVE 2	2	8	14.25892	false	8
72	CURVE 2	2	8	6.93715	false	8

	Column	Plate
1		2
2		3
3		4
4		2
5		3
6		4
7		2
8		3
9		4
10		2
11		3
12		4
13		2
14		3
15		4
16		2
17		3
18		4
19		2
20		3
21		4
22		2
23		3
24		4
25		5
26		6
27		7
28		5
29		6
30		7

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	Column	Plate
31	5	1
32	6	1
33	7	1
34	5	1
35	6	1
36	7	1
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71	9	1
72	10	1

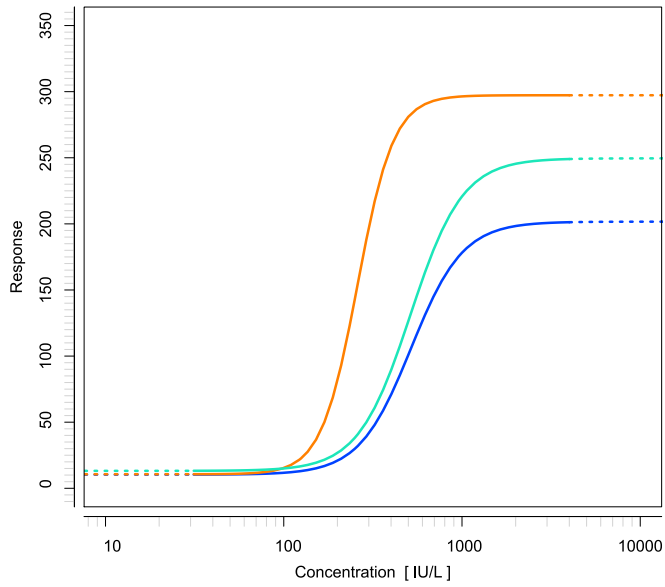
Assay suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	3	0	0	0
Overall test result	Passed			

<p>CURVE 1 F-Test (hypothesis test): Model Scope: all assay elements; Severity level: warning</p>		Passed
Calculated F-value	1000.3914	
F _{critical} (95%)	0.11547	
<p>CURVE 2 F-Test (hypothesis test): Model Scope: all assay elements; Severity level: warning</p>		Passed
Calculated F-value	2972.9291	
F _{critical} (95%)	0.11547	
<p>CURVE 3 F-Test (hypothesis test): Model Scope: all assay elements; Severity level: warning</p>		Passed
Calculated F-value	2017.0350	
F _{critical} (95%)	0.11547	

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Regression results overview



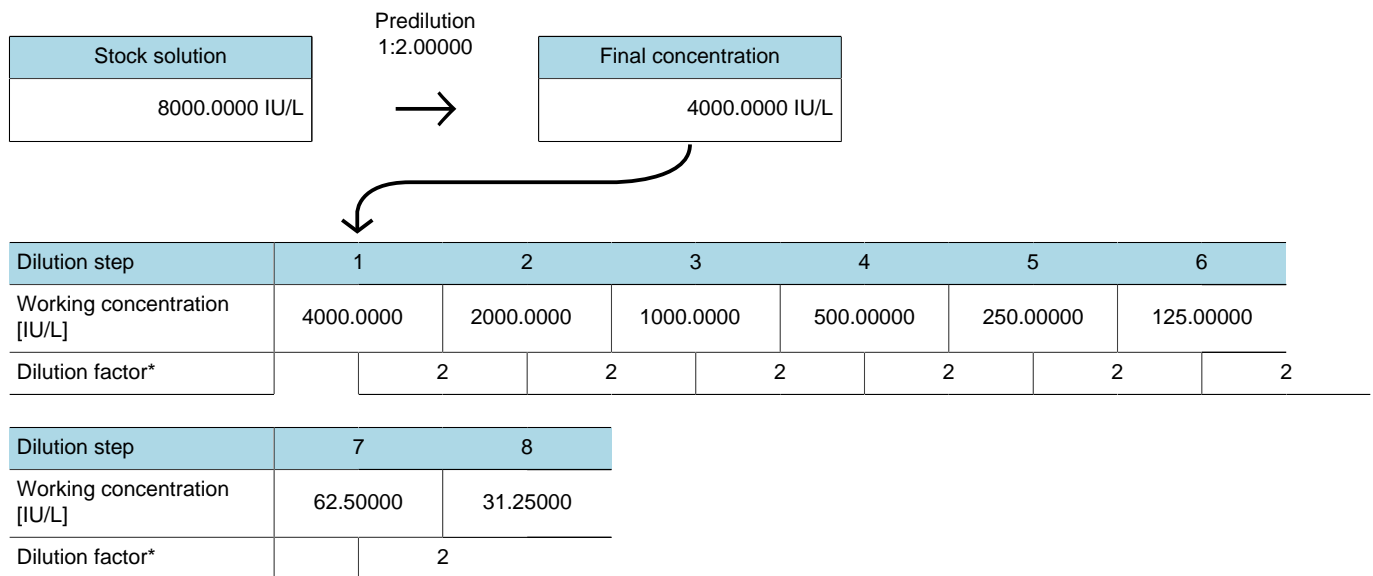
Assay elements	Suitability test status
CURVE 1	Passed
CURVE 2	Failed (warning)
CURVE 3	Passed

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CURVE 1: Setup

Property	Value
Concentration: Defined by stock solution	8000.0000 IU/L
Preparation scheme	MultiDoseScheme-1
Number of replicates	3
Predilution factors	2
Geometric Sequence	2-fold sequence
Number of steps	8
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

Working concentration



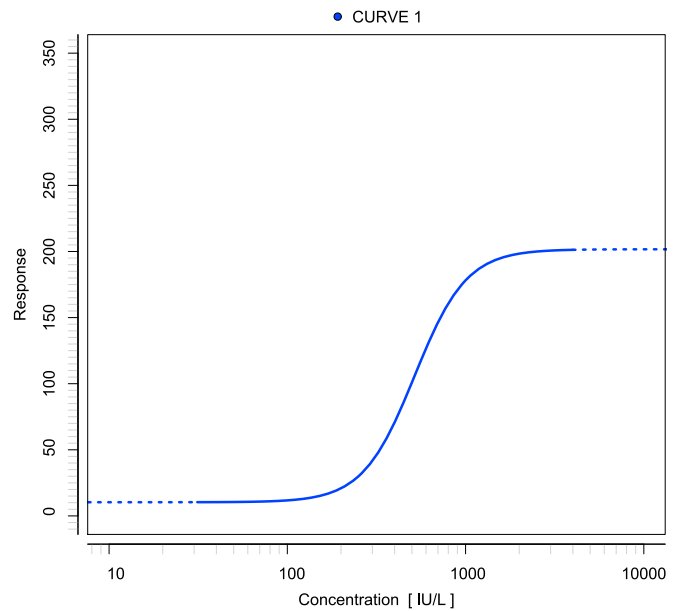
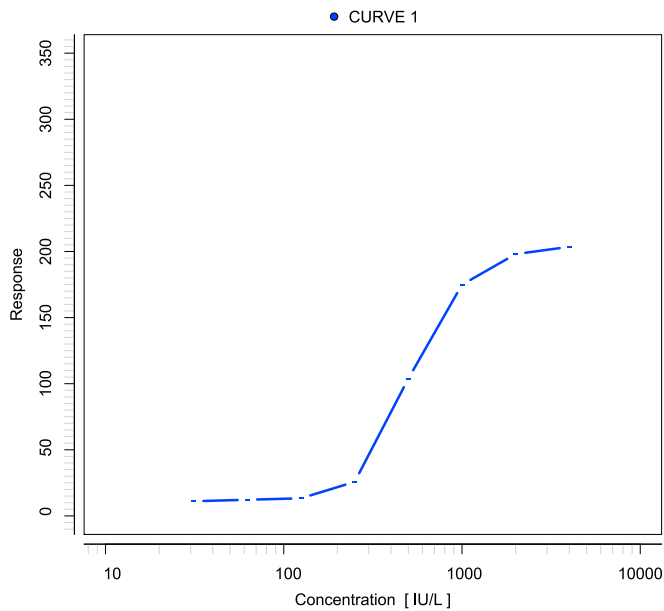
* Dilution factors are defined by the user.

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CURVE 1: Result

Data plot

Regression plot



Response data

Dilution step	1	2	3	4	5	6
Working concentration [IU/L]	4000.0000	2000.0000	1000.0000	500.00000	250.00000	125.00000
Response	211.91351 201.57012 197.06491	199.79343 195.20899 199.13072	177.24509 172.29252 175.07573	103.47476 104.81782 103.20709	17.17979 26.28608 33.82634	2.53044 12.83126 24.59858
Mean response	203.51618	198.04438	174.87111	103.83322	25.76407	13.32009
SD	7.61318	2.47778	2.48262	0.86312	8.33555	11.04218
CV	3.74%	1.25%	1.42%	0.83%	32.35%	82.90%

Dilution step	7	8
Working concentration [IU/L]	62.50000	31.25000
Response	16.87861 1.75747 18.00377	23.64852 1.28774 8.34130
Mean response	12.21328	11.09252
SD	9.07246	11.43145
CV	74.28%	103.06%

Analysis of variance (ANOVA)

Source of variation	d.f.	Sum of squares	Mean squares	F-ratio	Probability
Model	3	162387.79	54129.264	1000.3914	5.963 E-22
Residual error	20	1082.1617	54.10809		
Lack of fit	4	131.34765	32.83691	0.55257	0.70003
Pure error	16	950.81409	59.42588		
Total	23	163469.95	7107.3893		

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Regression results

Parameter	Estimate	Standard error	Confidence interval (95%)	Width of CI
A upper asymptote	201.67853	3.34665	194.69754 to 208.65952	6.92%
B parameter	2.98947	0.28139	2.40249 to 3.57644	39.27%
C parameter	9.01692	0.04702	8.91884 to 9.11500	2.18%
D lower asymptote	10.34121	2.58468	4.94966 to 15.73276	104.27%
<i>Curve characteristics</i>				
A/D ratio of asymptotes	19.50241	4.56330	-117.55188 to 171.28128	1481.01%
A-D difference of asymptotes	191.33732	4.56330	181.81844 to 200.85620	9.95%
EC50	518.03913	N/A	483.99096 to 554.48254	13.61%

Quality of regression	
r ²	0.99338
r ² adjusted	0.99239
Iterations	34
Final tolerance	Reduced accuracy 1.490 E-08
Reason for termination	Relative error in the sum of squares is at most `ftol'.

Covariance

	Parameter A	Parameter B	Parameter C	Parameter D
Parameter A	11.20006	-0.50141	0.07308	-1.47154
Parameter B	-0.50141	0.07918	-0.00159	0.31703
Parameter C	0.07308	-0.00159	0.00221	0.03766
Parameter D	-1.47154	0.31703	0.03766	6.68058

Effective concentration (EC)

	5%	20%	50%	80%	95%
Percentile EC [IU/L]	193.46829	325.81340	518.03913	823.67558	1387.1241
Response at EC	19.90808	48.60868	106.00987	163.41106	192.11166

Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	3	0	0	0
Sample suitability tests	3	0	0	0
Overall test result	Passed			

<p>■ CURVE 1 Test: Parameter/Property point estimate, Parameter: A/D ratio of asymptotes Scope: all assay elements; Severity level: warning</p>		Passed
Calculated value	19.50241	
Margins	10.00000 to 25.00000	
<p>■ CURVE 1 Test: Mean squares (ANOVA): Residual Error Scope: all assay elements; Severity level: warning</p>		Passed
Calculated value	54.10809	
Upper margin	64.00000	
<p>■ CURVE 1 Test: Sum of squares (ANOVA): Lack of fit Scope: all assay elements; Severity level: warning</p>		Passed
Calculated value	131.34765	
Upper margin	256.00000	

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CURVE 2: Setup

Property	Value
Preparation scheme	MultiDoseScheme-2
Number of replicates	3
Stepwise concentration sequence	4000, 2000, 1000, 500, 250, 125, 62.5, 31.25
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

Working concentration

Dilution step	1	2	3	4	5	6
Working concentration	4000.0000	2000.0000	1000.0000	500.00000	250.00000	125.00000
Dilution factor*		2.00000	2.00000	2.00000	2.00000	2.00000

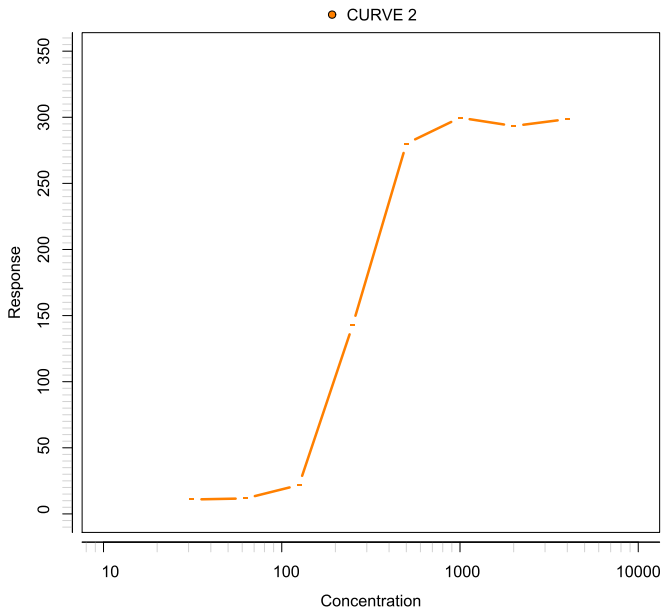
Dilution step	7	8
Working concentration	62.50000	31.25000
Dilution factor*	2.00000	

* Dilution factors are calculated by PLA.

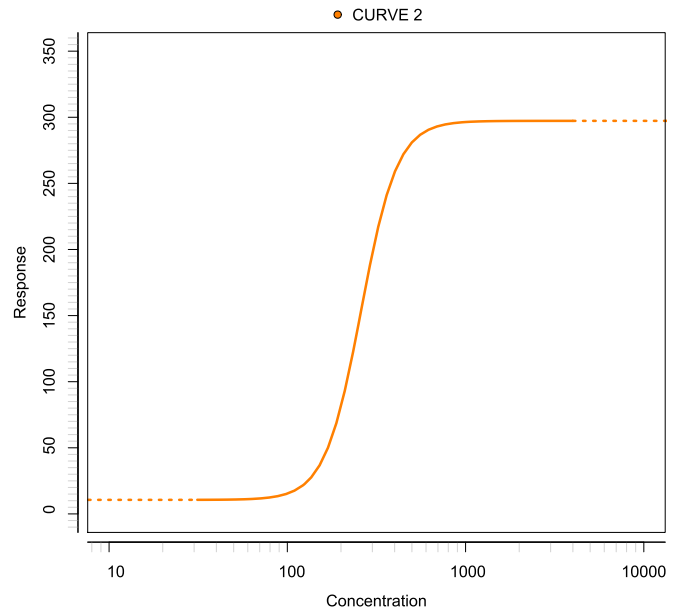
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CURVE 2: Result

Data plot



Regression plot



Response data

Dilution step	1	2	3	4	5	6
Working concentration	4000.0000	2000.0000	1000.0000	500.00000	250.00000	125.00000
Response	292.56633 295.98557 307.28481	304.10191 282.55916 293.54266	307.14955 296.29104 295.61676	283.19310 286.59337 270.46342	133.84735 144.47419 150.09980	19.20772 24.55355 21.67286
Mean response	298.61224	293.40124	299.68578	280.08330	142.80711	21.81137
SD	7.70279	10.77207	6.47260	8.50276	8.25348	2.67560
CV	2.58%	3.67%	2.16%	3.04%	5.78%	12.27%

Dilution step	7	8
Working concentration	62.50000	31.25000
Response	17.29848 6.86793 10.90535	11.51796 14.25892 6.93715
Mean response	11.69059	10.90468
SD	5.25942	3.69921
CV	44.99%	33.92%

Analysis of variance (ANOVA)

Source of variation	d.f.	Sum of squares	Mean squares	F-ratio	Probability
Model	3	401246.89	133748.96	2972.9291	1.162 E-26
Residual error	20	899.77903	44.98895		
Lack of fit	4	87.40599	21.85150	0.43037	0.78465
Pure error	16	812.37304	50.77331		
Total	23	402146.66	17484.638		

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Regression results

Parameter	Estimate	Standard error	Confidence interval (95%)	Width of CI
A upper asymptote	297.28356	2.23964	292.61175 to 301.95536	3.14%
B parameter	4.28465	0.34888	3.55689 to 5.01240	33.97%
C parameter	8.01963	0.02032	7.97723 to 8.06202	1.06%
D lower asymptote	10.62306	2.57761	5.24627 to 15.99986	101.23%
<i>Curve characteristics</i>				
A/D ratio of asymptotes	27.98473	3.65788	-164.63252 to 239.96774	1445.79%
A-D difference of asymptotes	286.66049	3.65788	279.03028 to 294.29070	5.32%
EC50	259.50660	N/A	251.99133 to 267.24600	5.88%
EC50 Ratio	0.50094			

Quality of regression	
r ²	0.99776
r ² adjusted	0.99743
Iterations	33
Final tolerance	Reduced accuracy 1.490 E-08
Reason for termination	Relative error in the sum of squares is at most `ftol`.

Covariance

	Parameter A	Parameter B	Parameter C	Parameter D
Parameter A	5.01599	-0.33381	0.01509	-0.86003
Parameter B	-0.33381	0.12172	-8.132 E-04	0.37308
Parameter C	0.01509	-8.132 E-04	4.131 E-04	0.01537
Parameter D	-0.86003	0.37308	0.01537	6.64405

Effective concentration (EC)

	5%	20%	50%	80%	95%
Percentile EC	130.52638	187.77284	259.50660	358.64438	515.93916
Response at EC	24.95609	67.95516	153.95331	239.95146	282.95053

Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	3	0	0	0
Sample suitability tests	2	0	1	0
Overall test result	Failed (warning)			

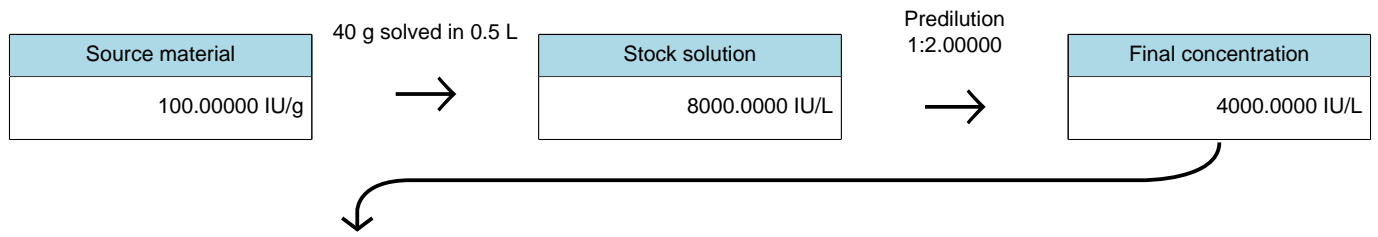
<p>■ CURVE 2 Test: Parameter/Property point estimate, Parameter: A/D ratio of asymptotes Scope: all assay elements; Severity level: warning</p>		Failed
Calculated value	27.98473	
Margins	10.00000 to 25.00000	
<p>■ CURVE 2 Test: Mean squares (ANOVA): Residual Error Scope: all assay elements; Severity level: warning</p>		Passed
Calculated value	44.98895	
Upper margin	64.00000	
<p>■ CURVE 2 Test: Sum of squares (ANOVA): Lack of fit Scope: all assay elements; Severity level: warning</p>		Passed
Calculated value	87.40599	
Upper margin	256.00000	

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■ CURVE 3: Setup

Property	Value
Concentration: Defined by source material	100.00000 IU/g
Preparation scheme	MultiDoseScheme-3
Number of replicates	3
Predilution factors	2
Geometric Sequence	2-fold sequence
Number of steps	8
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

Working concentration



Dilution step	1	2	3	4	5	6
Working concentration [IU/L]	4000.0000	2000.0000	1000.0000	500.00000	250.00000	125.00000
Dilution factor*		2	2	2	2	2

Dilution step	7	8
Working concentration [IU/L]	62.50000	31.25000
Dilution factor*		2

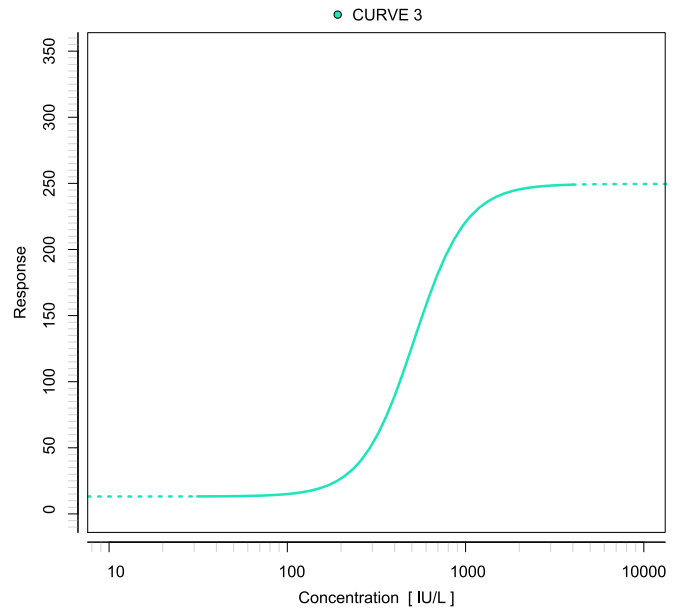
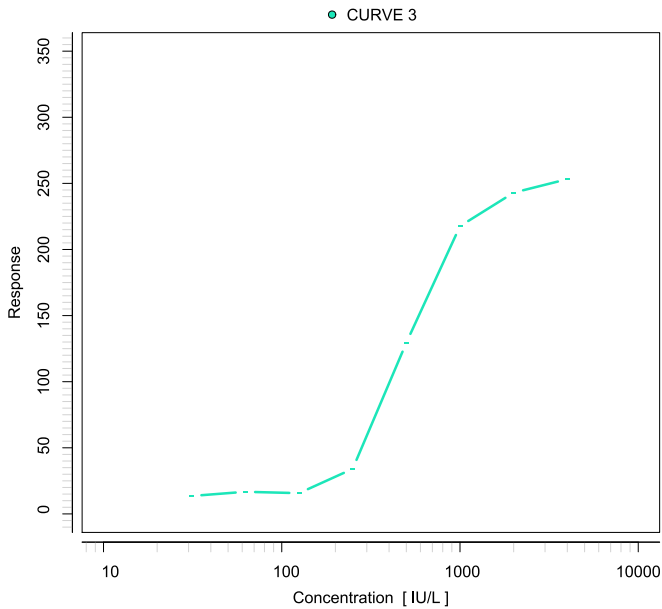
* Dilution factors are defined by the user.

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CURVE 3: Result

Data plot

Regression plot



Response data

Dilution step	1	2	3	4	5	6
Working concentration [IU/L]	4000.0000	2000.0000	1000.0000	500.00000	250.00000	125.00000
Response	250.19531 250.70353 258.68016	247.79815 232.95436 248.16306	216.72223 210.83509 225.38977	125.05083 126.28014 137.13765	28.80068 39.18680 34.20951	16.37005 19.97453 10.73337
Mean response	253.19300	242.97186	217.64903	129.48954	34.06566	15.69265
SD	4.75881	8.67732	7.32147	6.65192	5.19455	4.65767
CV	1.88%	3.57%	3.36%	5.14%	15.25%	29.68%

Dilution step	7	8
Working concentration [IU/L]	62.50000	31.25000
Response	14.30931 20.65082 15.22821	6.01348 14.71754 19.97839
Mean response	16.72944	13.56980
SD	3.42695	7.05285
CV	20.48%	51.97%

Analysis of variance (ANOVA)

Source of variation	d.f.	Sum of squares	Mean squares	F-ratio	Probability
Model	3	247524.69	82508.231	2017.0350	5.561 E-25
Residual error	20	818.11402	40.90570		
Lack of fit	4	206.19810	51.54953	1.34789	0.29546
Pure error	16	611.91591	38.24474		
Total	23	248342.81	10797.513		

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Regression results

Parameter	Estimate	Standard error	Confidence interval (95%)	Width of CI
A upper asymptote	249.57541	2.90828	243.50885 to 255.64197	4.86%
B parameter	2.96754	0.19593	2.55883 to 3.37625	27.55%
C parameter	9.00610	0.03327	8.93671 to 9.07550	1.54%
D lower asymptote	13.18506	2.25901	8.47286 to 17.89726	71.48%
<i>Curve characteristics</i>				
A/D ratio of asymptotes	18.92865	3.97669	-80.53195 to 123.99737	1080.53%
A-D difference of asymptotes	236.39035	3.97669	228.09512 to 244.68558	7.02%
EC50	514.17090	N/A	490.02536 to 539.50620	9.62%
EC50 Ratio	0.99253			

Quality of regression	
r ²	0.99671
r ² adjusted	0.99621
Iterations	34
Final tolerance	Reduced accuracy 1.490 E-08
Reason for termination	Relative error in the sum of squares is at most `ftol`.

Covariance

	Parameter A	Parameter B	Parameter C	Parameter D
Parameter A	8.45808	-0.30307	0.04474	-1.12644
Parameter B	-0.30307	0.03839	-7.368 E-04	0.19477
Parameter C	0.04474	-7.368 E-04	0.00111	0.02358
Parameter D	-1.12644	0.19477	0.02358	5.10310

Effective concentration (EC)

	5%	20%	50%	80%	95%
Percentile					
EC [IU/L]	190.63113	322.27428	514.17090	820.33141	1386.8235
Response at EC	25.00458	60.46313	131.38023	202.29734	237.75589

Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	3	0	0	0
Sample suitability tests	3	0	0	0
Overall test result	Passed			

<p>CURVE 3 Test: Parameter/Property point estimate, Parameter: A/D ratio of asymptotes Scope: all assay elements; Severity level: warning</p>		Passed
Calculated value	18.92865	
Margins	10.00000 to 25.00000	
<p>CURVE 3 Test: Mean squares (ANOVA): Residual Error Scope: all assay elements; Severity level: warning</p>		Passed
Calculated value	40.90570	
Upper margin	64.00000	
<p>CURVE 3 Test: Sum of squares (ANOVA): Lack of fit Scope: all assay elements; Severity level: warning</p>		Passed
Calculated value	206.19810	
Upper margin	256.00000	