

## Appendix 8.3 Data sheet format

- 1) Raw data
- 2) Run1
- 3) Run2
- 4) Run3
- 5) Summary

# SIRC validation study datasheet

Study director
Mai Endo

Test substance	AAA2015
Code of Test Substance	03
Relative control substance	Triethanolamine
Positive control substance	Sodium Dodecyl Sulfate
Negative control substance	DMSO

Total nums of runs	2	Run1	1	Run2	2	Run3	
Cell seeding day (yyyy/mm/dd)		2013/05/27		2015/05/31			
Name of experimenter		Takashi Omori		Yumi Takaka			
Passage No. at the time of purchase		1		1			
Passage No. after thawing		4		4			
Passage No. at the time of assay		6		7			
Test substance conc.(%)		1.000		1.000			
Relative control substance conc.(%)		1.000		1.000			
Positive control substance conc.(%)		0.100		0.100			

Run1	1											
0.056	0.070	0.094	0.109	0.073	0.086	0.086	0.205	0.050	0.060	0.069	0.127	
0.109	0.551					0.543	0.511	0.422	0.586	0.501	0.592	0.128
0.074	0.594					0.439	0.556	0.562	0.452	0.522	0.547	0.143
0.072	0.555	0.066	0.080	0.336	0.526	0.584	0.489	0.448	0.568	0.527	0.072	
0.095	0.602	0.064	0.092	0.356	0.414	0.435	0.437	0.422	0.594	0.526	0.092	
0.072	0.514	0.059	0.100	0.076	0.050	0.051	0.114	0.429	0.530	0.512	0.116	
0.066	0.509	0.066	0.071	0.110	0.063	0.063	0.105	0.561	0.536	0.524	0.116	
0.068	0.124	0.092	0.098	0.110	0.056	0.092	0.068	0.136	0.116	0.099	0.052	
Test substance		Relative control substance			Positive control substance			Negative control substance				

Run2	2										
0.093	0.084	0.09	0.066	0.053	0.056	0.054	0.061	0.062	0.051	0.134	0.092
0.056	0.533	0.539	0.522	0.588	0.537	0.536	0.593	0.575	0.582	0.453	0.065
0.057	0.526	0.461	0.451	0.578	0.556	0.591	0.582	0.415	0.402	0.481	0.064
0.079	0.581	0.065	0.096	0.461	0.534	0.546	0.525	0.51	0.529	0.498	0.066
0.071	0.468	0.078	0.0621	0.222	0.683	0.548	0.508	0.597	0.505	0.539	0.056
0.067	0.579	0.054	0.056	0.055	0.060	0.064	0.255	0.356	0.653	0.513	0.068
0.091	0.585	0.044	0.043	0.051	0.072	0.063	0.153	0.347	0.579	0.418	0.067
0.078	0.11	0.076	0.086	0.066	0.056	0.108	0.052	0.122	0.068	0.099	0.072
Test substance			Relative control substance			Positive control substance			Negative control substance		

Run3											
0.055	0.054	0.056	0.058	0.059	0.055	0.054	0.053	0.057	0.062	0.053	0.052
0.051	1.065	0.341	0.623	0.427	0.318	0.635	0.778	0.878	0.901	1.129	0.051
0.054	1.088	0.257	0.584	0.466	0.343	0.638	0.797	0.837	0.808	1.154	0.052
0.05	0.998	0.061	0.08	0.598	0.988	1.091	1.129	1.1	1.115	1.164	0.051
0.052	1.015	0.068	0.078	0.715	0.872	1.029	1.056	1.079	1.176	1.065	0.052
0.055	1.047	0.134	0.056	0.052	1.169	1.152	1.183	1.182	1.152	1.141	0.053
0.055	1.032	0.141	0.058	0.052	1.281	1.174	1.184	1.146	1.092	1.116	0.054
0.055	0.056	0.053	0.054	0.054	0.054	0.053	0.054	0.052	0.05	0.052	0.054
Test substance			Relative control substance			Positive control substance			Negative control substance		

Comment:

[illegible]

Sub_exp.1	1										
0.056	0.07	0.094	0.109	0.073	0.086	0.086	0.205	0.05	0.06	0.069	0.127
0.109	0.551	0	0	0	0.543	0.511	0.422	0.586	0.501	0.592	0.128
0.074	0.594	0	0	0	0.439	0.556	0.562	0.452	0.522	0.547	0.143
0.072	0.555	0.066	0.08	0.336	0.526	0.584	0.489	0.448	0.568	0.527	0.072
0.095	0.602	0.064	0.092	0.356	0.414	0.435	0.437	0.422	0.594	0.526	0.092
0.072	0.514	0.059	0.1	0.076	0.05	0.051	0.114	0.429	0.53	0.512	0.116
0.066	0.509	0.066	0.071	0.11	0.063	0.063	0.105	0.561	0.536	0.524	0.116
0.068	0.124	0.092	0.098	0.11	0.056	0.092	0.068	0.136	0.116	0.099	0.052

(a) Blank	0.093
(b) Mean OD of negative controls (left side)	0.554
(c) Mean OD of negative controls (right side)	0.538
(d) Mean OD of both negative controls	0.546
(e) Negative control (l) - Blank [(b) - (a)]	0.461
(f) Negative control (r) - Blank [(c) - (a)]	0.445
(g) Standard deviation of OD for negative controls	0.034
(h) 15% mean of negative controls [(d) * 0.15]	0.082
(i) Mean OD of negative controls+15% [(d) + (h)]	0.628
(j) Mean OD of negative controls -15% [(d) - (h)]	0.464

Test substance	AAA2015					
Prepared solution (%)	1.0		Common ratio	2	Final maximum conc. (µg/mL)	5000

Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
5000	0	0	0	-20.5	Low	625 87.8
2500	0	0	0	-20.5	High	1250 -20.5
1250	0	0	0	-20.5		
625	0.543	0.439	0.491	87.8	IC50	Not estimated µg/mL
312.5	0.511	0.556	0.534	97.2		
156.3	0.422	0.562	0.492	88.1	Data1 Data2 Mean	
78.1	0.586	0.452	0.519	94.0	Not es! Not es! Not estimated	
39.1	0.501	0.522	0.512	92.4		

Relative control substance					
Prepared solution (%)	1.0	Common ratio	2	Final maximum conc. (µg/mL)	5000

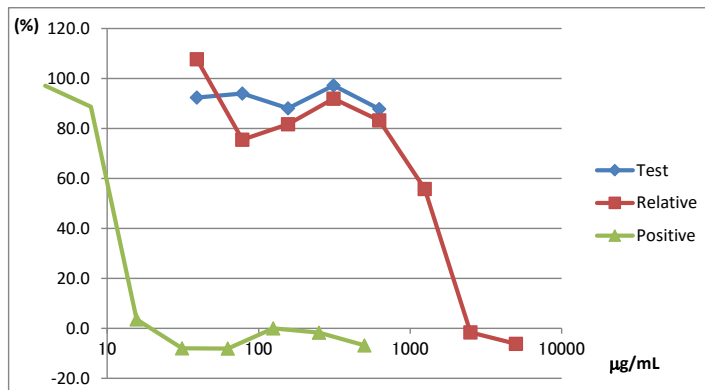
Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
5000	0.066	0.064	0.065	-6.2	Low	1250.0 55.8
2500	0.08	0.092	0.086	-1.6	High	2500.0 -1.6
1250	0.336	0.356	0.346	55.8		
625	0.526	0.414	0.47	83.2	IC50	1,340.7 µg/mL
312.5	0.584	0.435	0.51	91.9		
156.3	0.489	0.437	0.463	81.7		
78.1	0.448	0.422	0.435	75.5		
39.1	0.568	0.594	0.581	107.7		

Positive control substance					
Prepared solution (%)	0.1	Common ratio	2	Final maximum conc. (µg/mL)	500

Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
500	0.059	0.066	0.063	-6.8	Low	7.8 88.7
250	0.1	0.071	0.086	-1.7	High	15.6 3.6
125	0.076	0.11	0.093	0.0		
62.5	0.05	0.063	0.057	-8.1	IC50	10.7 µg/mL
31.25	0.051	0.063	0.057	-8.0		
15.63	0.114	0.105	0.11	3.6		
7.8	0.429	0.561	0.495	88.7		
3.9	0.53	0.536	0.533	97.1		

QC check	Judgement
(1) 0.546	OK
(2) 10.7	Retest
(3) 1,340.7	OK
(4)M Not estim	Retest
(4)1 Not estim	(Retest)
(4)2 Not estim	(Retest)
(5)M 0.546	OK
(5)L 0.554	(OK)
(5)R 0.538	(OK)

Conc.	Test	Relativ	Positive
5000	#N/A	#N/A	#N/A
2500	#N/A	#N/A	#N/A
1250	#N/A	#N/A	#N/A
625	87.8	#N/A	#N/A
312.5	97.2	#N/A	#N/A
156.3	88.1	#N/A	#N/A
78.1	94.0	#N/A	#N/A
39.1	92.4	#N/A	#N/A
500.0	#N/A	#N/A	-6.8
250.0	#N/A	#N/A	-1.7
125.0	#N/A	#N/A	0.0
62.5	#N/A	#N/A	-8.1
31.3	#N/A	#N/A	-8.0
15.6	#N/A	#N/A	3.6
7.8	#N/A	#N/A	88.7
3.9	#N/A	#N/A	97.1
5000	#N/A	-6.2	#N/A
2500	#N/A	-1.6	#N/A
1250	#N/A	55.8	#N/A
625	#N/A	83.2	#N/A
313	#N/A	91.9	#N/A
156	#N/A	81.7	#N/A
78	#N/A	75.5	#N/A
39	#N/A	107.7	#N/A



Sub_exp.2	2										
0.093	0.084	0.09	0.066	0.053	0.056	0.054	0.061	0.062	0.051	0.134	0.092
0.056	0.533	0.539	0.522	0.588	0.537	0.536	0.593	0.575	0.582	0.453	0.065
0.057	0.526	0.461	0.451	0.578	0.556	0.591	0.582	0.415	0.402	0.481	0.064
0.079	0.581	0.065	0.096	0.461	0.534	0.546	0.525	0.51	0.529	0.498	0.066
0.071	0.468	0.078	0.062	0.222	0.683	0.548	0.508	0.597	0.505	0.539	0.056
0.067	0.579	0.054	0.056	0.055	0.06	0.064	0.255	0.356	0.653	0.513	0.068
0.091	0.585	0.044	0.043	0.051	0.072	0.063	0.153	0.347	0.579	0.418	0.067
0.078	0.11	0.076	0.086	0.066	0.056	0.108	0.052	0.122	0.068	0.099	0.072

(a) Blank	0.075
(b) Mean OD of negative controls (left side)	0.545
(c) Mean OD of negative controls (right side)	0.484
(d) Mean OD of both negative controls	0.515
(e) Negative control (l) - Blank [(b) - (a)]	0.47
(f) Negative control (r) - Blank [(c) - (a)]	0.409
(g) Standard deviation of OD for negative controls	0.053
(h) 15% mean of negative controls [(d) * 0.15]	0.077
(i) Mean OD of negative controls+15% [(d) + (h)]	0.592
(j) Mean OD of negative controls -15% [(d) - (h)]	0.437

Test substance	AAA2015					
Prepared solution (%)	1.0		Common ratio	2	Final maximum conc. (µg/mL)	5000

Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
5000	0.539	0.461	0.5	96.7	Low	0.0 #N/A
2500	0.522	0.451	0.487	93.6	High	0.0 #N/A
1250	0.588	0.578	0.583	115.6		
625	0.537	0.556	0.547	107.3	IC50	>5000 µg/mL
312.5	0.536	0.591	0.563	111.1		
156.3	0.593	0.582	0.588	116.6	Data1	Data2
78.1	0.575	0.415	0.495	95.6	5,000	5,000
39.1	0.582	0.402	0.492	94.9		5,000

Relative control substance					
Prepared solution (%)	1.0	Common ratio	2	Final maximum conc. (µg/mL)	5000

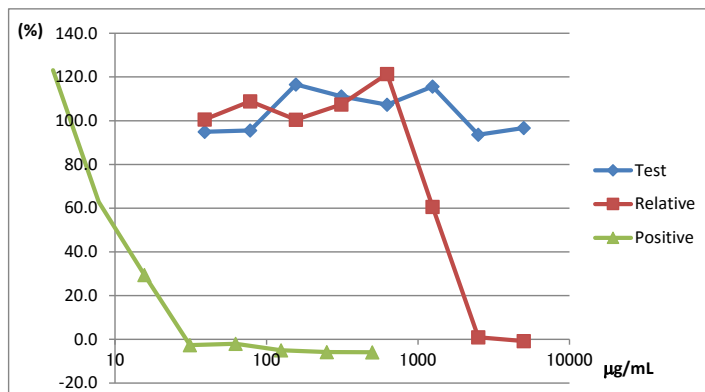
Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
5000	0.065	0.078	0.072	-0.8	Low	1250.0 60.6
2500	0.096	0.062	0.079	0.9	High	2500.0 0.9
1250	0.461	0.222	0.342	60.6		
625	0.534	0.683	0.609	121.4	IC50	1,413.7 µg/mL
312.5	0.546	0.548	0.547	107.4		
156.3	0.525	0.508	0.517	100.5		
78.1	0.51	0.597	0.554	108.9		
39.1	0.529	0.505	0.517	100.6		

Positive control substance					
Prepared solution	(%)	0.1	Common ratio	2	Final maximum conc. (µg/mL)
					500

Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
500	0.054	0.044	0.049	-5.9	Low	7.8 62.9
250	0.056	0.043	0.05	-5.8	High	15.6 29.4
125	0.055	0.051	0.053	-5.0		
62.5	0.06	0.072	0.066	-2.0	IC50	10.2 µg/mL
31.25	0.064	0.063	0.064	-2.6		
15.63	0.255	0.153	0.204	29.4		
7.8	0.356	0.347	0.352	62.9		
3.9	0.653	0.579	0.616	123.1		

QC check	Judgement
(1) 0.515	OK
(2) 10.2	Retest
(3) 1,413.7	OK
(4)M 5,000.0	OK
(4)1 5,000.0	(OK)
(4)2 5,000.0	(OK)
(5)M 0.515	OK
(5)L 0.545	(OK)
(5)R 0.484	(OK)

Conc.	Test	Relativ	Positive
5000	96.7	#N/A	#N/A
2500	93.6	#N/A	#N/A
1250	115.6	#N/A	#N/A
625	107.3	#N/A	#N/A
312.5	111.1	#N/A	#N/A
156.3	116.6	#N/A	#N/A
78.1	95.6	#N/A	#N/A
39.1	94.9	#N/A	#N/A
500.0	#N/A	#N/A	-5.9
250.0	#N/A	#N/A	-5.8
125.0	#N/A	#N/A	-5.0
62.5	#N/A	#N/A	-2.0
31.3	#N/A	#N/A	-2.6
15.6	#N/A	#N/A	29.4
7.8	#N/A	#N/A	62.9
3.9	#N/A	#N/A	123.1
5000	#N/A	-0.8	#N/A
2500	#N/A	0.9	#N/A
1250	#N/A	60.6	#N/A
625	#N/A	121.4	#N/A
313	#N/A	107.4	#N/A
156	#N/A	100.5	#N/A
78	#N/A	108.9	#N/A
39	#N/A	100.6	#N/A



Sub_exp.3	0											
0.055	0.054	0.056	0.058	0.059	0.055	0.054	0.053	0.057	0.062	0.053	0.052	
0.051	1.065	0.341	0.623	0.427	0.318	0.635	0.778	0.878	0.901	1.129	0.051	
0.054	1.088	0.257	0.584	0.466	0.343	0.638	0.797	0.837	0.808	1.154	0.052	
0.05	0.998	0.061	0.08	0.598	0.988	1.091	1.129	1.1	1.115	1.164	0.051	
0.052	1.015	0.068	0.078	0.715	0.872	1.029	1.056	1.079	1.176	1.065	0.052	
0.055	1.047	0.134	0.056	0.052	1.169	1.152	1.183	1.182	1.152	1.141	0.053	
0.055	1.032	0.141	0.058	0.052	1.281	1.174	1.184	1.146	1.092	1.116	0.054	
0.055	0.056	0.053	0.054	0.054	0.054	0.053	0.054	0.052	0.05	0.052	0.054	

(a) Blank	0.054
(b) Mean OD of negative controls (left side)	1.041
(c) Mean OD of negative controls (right side)	1.128
(d) Mean OD of both negative controls	1.085
(e) Negative control (l) - Blank [(b) - (a)]	0.987
(f) Negative control (r) - Blank [(c) - (a)]	1.074
(g) Standard deviation of OD for negative controls	0.056
(h) 15% mean of negative controls [(d) * 0.15]	0.163
(i) Mean OD of negative controls+15% [(d) + (h)]	1.247
(j) Mean OD of negative controls -15% [(d) - (h)]	0.922

Test substance	AAA2015					
Prepared solution (%)	0.0		Common ratio	2	Final maximum conc. (µg/mL)	0

Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
0	0.341	0.257	0.299	23.8	Low	0.0 23.8
0	0.623	0.584	0.604	53.3	High	0.0 23.8
0	0.427	0.466	0.447	38.1		
0	0.318	0.343	0.331	26.8	IC50	#NUM! µg/mL
0	0.635	0.638	0.637	56.5		
0	0.778	0.797	0.788	71.2	Data1 Data2 Mean	
0.0	0.878	0.837	0.858	78.0	#NUM! #NUM! #NUM!	
0.0	0.901	0.808	0.855	77.7		

Relative control substance					
Prepared solution (%)	0.0	Common ratio	2	Final maximum conc. (µg/mL)	0

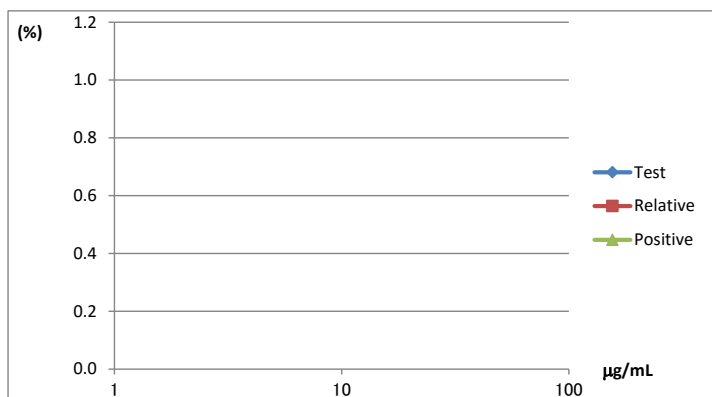
Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
0	0.061	0.068	0.065	1.0	Low	0.0 1.0
0	0.08	0.078	0.079	2.4	High	0.0 1.0
0	0.598	0.715	0.657	58.5		
0	0.988	0.872	0.93	85.0	IC50	#NUM! µg/mL
0	1.091	1.029	1.06	97.6		
0	1.129	1.056	1.093	100.8		
0.0	1.1	1.079	1.09	100.5		
0.0	1.115	1.176	1.146	105.9		

Positive control substance				
Prepared solution (%)	0.0	Common ratio	2	Final maximum conc. (µg/mL)

Conc.	Data1	Data2	Mean	Cell Viability (%)	Conc.	Cell Viability (%)
0	0.134	0.141	0.138	8.1	Low	0.0 8.1
0	0.056	0.058	0.057	0.3	High	0.0 8.1
0	0.052	0.052	0.052	-0.2		
0	1.169	1.281	1.225	113.6	IC50	#NUM! µg/mL
0	1.152	1.174	1.163	107.6		
0	1.183	1.184	1.184	109.6		
0.0	1.182	1.146	1.164	107.7		
0.0	1.152	1.092	1.122	103.6		

QC check	Judgement
(1) 1.085	OK
(2) #####	#NUM!
(3) #NUM!	#NUM!
(4)M #NUM!	#NUM!
(4)1 #NUM!	#NUM!
(4)2 #NUM!	#NUM!
(5)M 1.085	OK
(5)L 1.041	(OK)
(5)R 1.128	(OK)

Conc.	Test	Relativ	Positive
0	23.8	#N/A	#N/A
0	53.3	#N/A	#N/A
0	38.1	#N/A	#N/A
0	26.8	#N/A	#N/A
0	56.5	#N/A	#N/A
0	71.2	#N/A	#N/A
0.0	78.0	#N/A	#N/A
0.0	77.7	#N/A	#N/A
0.0	#N/A	#N/A	8.1
0.0	#N/A	#N/A	0.3
0.0	#N/A	#N/A	-0.2
0.0	#N/A	#N/A	113.6
0.0	#N/A	#N/A	107.6
0.0	#N/A	#N/A	109.6
0.0	#N/A	#N/A	107.7
0.0	#N/A	#N/A	103.6
0	#N/A	1.0	#N/A
0	#N/A	2.4	#N/A
0	#N/A	58.5	#N/A
0	#N/A	85.0	#N/A
0	#N/A	97.6	#N/A
0	#N/A	100.8	#N/A
0	#N/A	100.5	#N/A
0	#N/A	105.9	#N/A

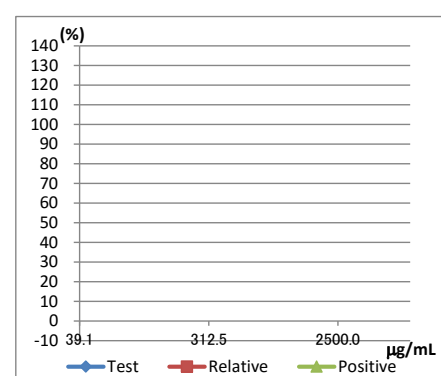
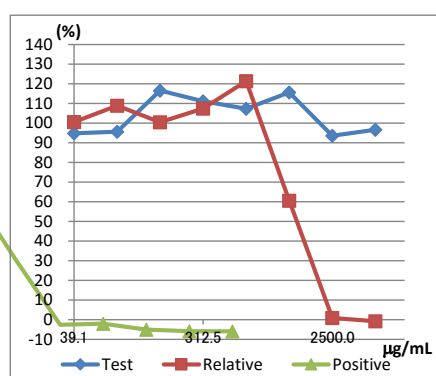


	IC50 (µg/mL)			Judgement	Final judgement
	Test substance	Relative control substance	Positive control substance		
Run1	Not estimated	1340.7	10.7	Not decided Negative	
Run2	>5000	1413.7	10.2		
Run3					

	(1)	(2)	(3)	(4)	(5)
Run1	OK 0.546	Retest 10.7	OK 1340.7	Retest Retest	OK 1.5% -1.5%
Run2	OK 0.515	Retest 10.2	OK 1413.7	OK 0.0% 0.0%	OK 6.0% -6.0%
Run3	OK 0.000				OK -4.0% 4.0%
Total	OK	Retest	OK	Retest	OK

(6)	
10.2 $\mu\text{g/mL}$ $\mu\text{g/mL}$	1.0
OK	

Concentration (μg/mL)	Test (%)	Relative (%)	Positive (%)
39.1	92	105	105
312.5	96	75	-10
2500.0	86	0	120



<b>Run1</b>	
Cell seeding day (yyyy/mm/dd)	
2013/05/27	
<b>Test substance</b>	
Test substance conc. (%)	1.0
Common ratio	2
Final maximum conc. (µg/mL)	5000
<b>Relative contorol substance</b>	
Relative contorol substance conc. (%)	1.0
Common ratio	2
Final maximum conc. (µg/mL)	5000
<b>Positive contorol substance</b>	
Positive contorol substance conc. (%)	0.1
Common ratio	2
Final maximum conc. (µg/mL)	500

Run2	
Cell seeding day (yyyy/mm/dd)	
2015/05/31	
Test substance	
Test substance conc. (%)	1.0
Common ratio	2
Final maximum conc. (μg/mL)	5000
Relative control substance	
Relative control substance conc. (%)	1.0
Common ratio	2
Final maximum conc. (μg/mL)	5000
Positive control substance	
Positive control substance conc. (%)	0.1
Common ratio	2
Final maximum conc. (μg/mL)	5000

Run3	
Cell seeding day (yyyy/mm/dd)	
0:00:00	
<b>Test substance</b>	
Test substance conc. (%)	0.0
Common ratio	2
Final maximum conc. (μg/mL)	0
<b>Relative control substance</b>	
Relative control substance conc. (%)	0.0
Common ratio	2
Final maximum conc. (μg/mL)	0
<b>Positive control substance</b>	
Positive control substance conc. (%)	0.0
Common ratio	2
Final maximum conc. (μg/mL)	

Comment: