Cytotoxicity Sensitivity Response in Epi-airway, Epi-gingival, Epi-vaginal, Epi-Intestinal Mucosa: Theraworx® Versus Chlorhexidine Gluconate.

The purpose of this testing is to compare the Cytoxicity of Theraworx® versus Dyna-Hex® a chlorhexidine gluconate formulation. MTT assays were used. The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport. Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light. The MTT assay is a colorimetric assay for assessing cell metabolic activity.

The clinical purpose for this study was to determine if Theraworx®, in comparision to CHG would be viable for mucosal therapy if proven to be non-cytoxic. The researchers selected airway mucosa (the most sensitive), vaginal, intestinal and gingival mucosas. Theraworx® in comparision to CHG was significantly less toxic to epi-airway, gingival, intestinal and vaginal mucosa. When considering managing macro and micro debris topical formulations have to have the best balance of potency and biocompatibility. Theraworx® is conluded to be a non-cytoxic formulation in mucous membrane tissues.

Tissue	Hours of Contact Before Seeing a Cytotoxic Response	
Epi-Airway	Theraworx®	5.07
	Dyna-Hex® (GHG)	0.05
Epi-Gingival	Theraworx®	399.36
	Dyna-Hex® (CHG)	8.86
Epi-Intestinal	Theraworx®	56.13
	Dyna-Hex®	1.42
Epi-Vaginal	Theraworx®	34.19
	Dyna-Hex®	6.16

Protocol #170118-407 Epi-Airway Tissues Test Date: 03/09/17

MTT ET- 50 in hours - Product 1 = 5.07

MTT ET- 50 in hours - Product2 = 0.01

MTT ET- 50 in hours - Positive Control = 0.00

Product #1 = Test Product : Broad Spectrum Hygiene Management (lot # 16180-1)
Product #2 = Reference Product : Dyna-Hex 2 (lot # 1021-912)
Positive Control = 1% Triton X-100 Soution Lot # 102016BBA exp 10-20-17
Negative Control = Sterile Water for Irrigation

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.039	0.038	0.072	0.066	0.071					1.720	1.759	1.823
B												
C												
D												
E		1.447	1.099	0.103	0.426	0.085	0.065					
F		1.826	1.305	0.276	0.432	0.080	0.071					
G		1.619	0.994	0.174	0.341	0.117	0.075					
H												

Endpoint
Lm1 570
Automix: Off Calibrate: On

Plate Last Read:
9:01 AM 3/10/2017

Wavelength Combination: !Lm1
Mean Temperature: 21.4
Data Mode: Absorbance

Product1 (Hours)

Sample	Wells	Time	Values	MeanValue	%Viability	CV%
01	E2	1	1.447	1.631	92.28	11.62
	F2		1.826			
	G2		1.619			
02	E3	4	1.099	1.133	64.09	14.00
	F3		1.305			
	G3		0.994			
03	E4	18	0.103	0.184	10.44	47.17
	F4		0.276			
	G4		0.174			

Product2 (hours)

Sample	Wells	Time	Values	MeanValue	%Viability	CV%
01	E5	1	0.426	0.400	22.61	12.81
	F5		0.432			
	G5		0.341			
02	E6	4	0.085	0.094	5.30	21.61
	F6		0.080			
	G6		0.117			
03	E7	18	0.065	0.070	3.97	7.34
	F7		0.071			
	G7		0.075			

PositiveControl (Hours)

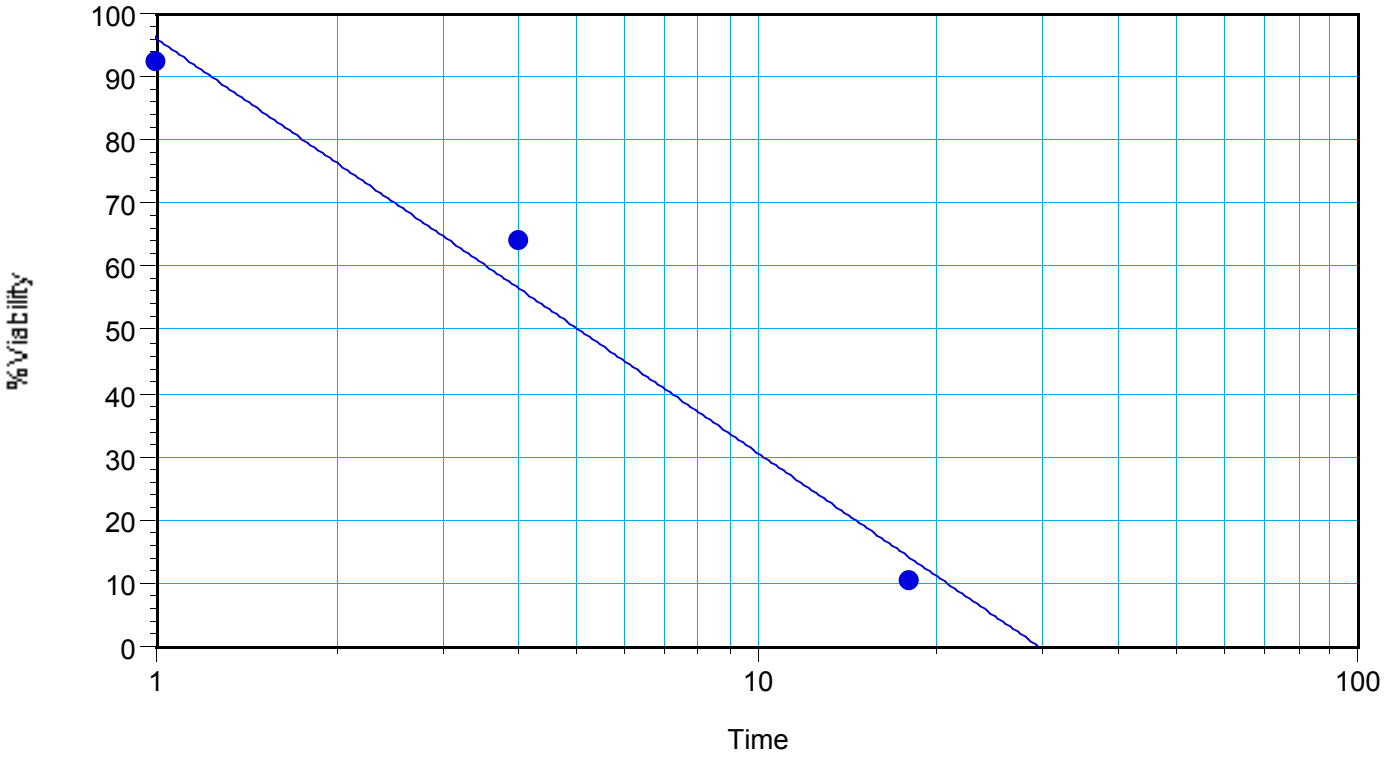
Sample	Wells	Time	Values	%Viability
01	A3	1	0.072	4.09
02	A4	2	0.066	3.74
03	A5	4	0.071	3.99

NegativeControl (Hours)

Sample	Wells	Time	Value
01	A10	4	1.720
	A11		1.759
	A12		1.823

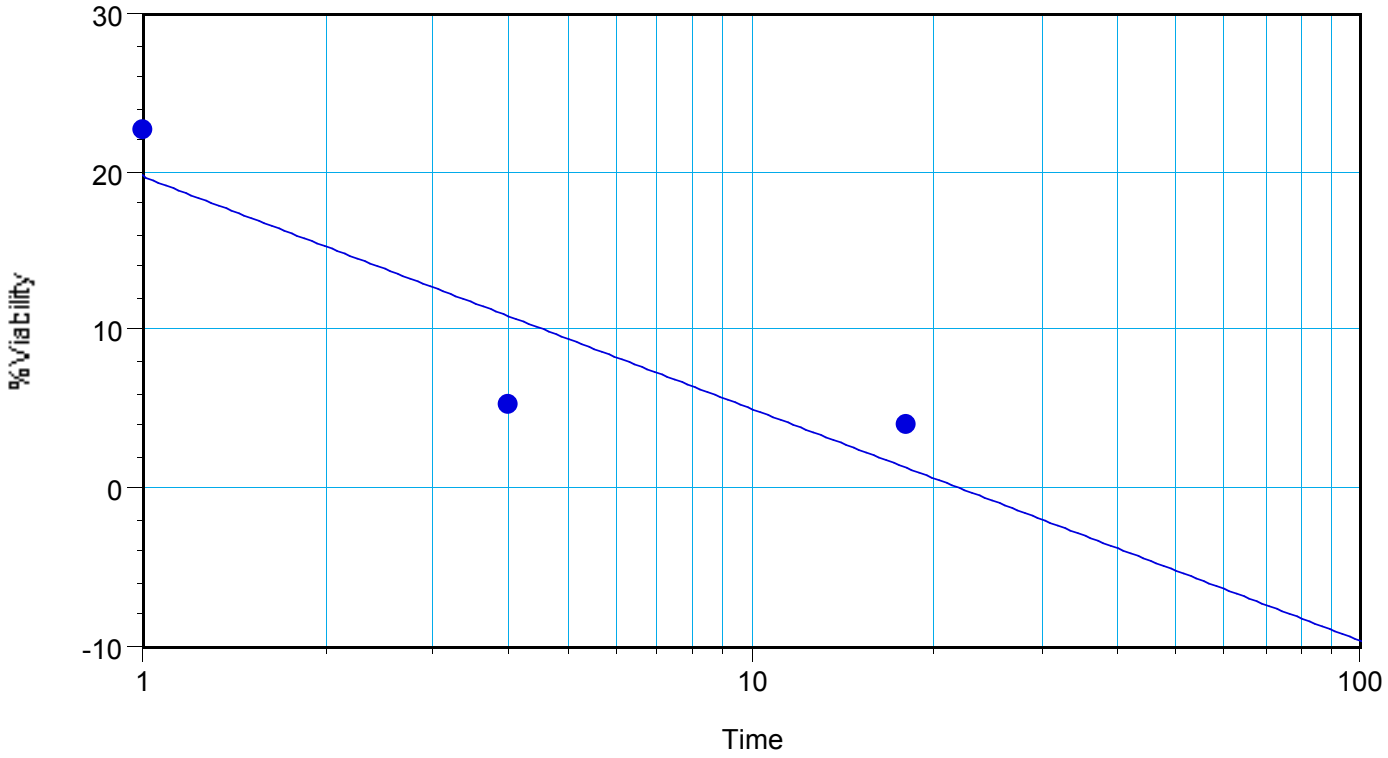
Negative Control Value = 1.767

Product1Graph



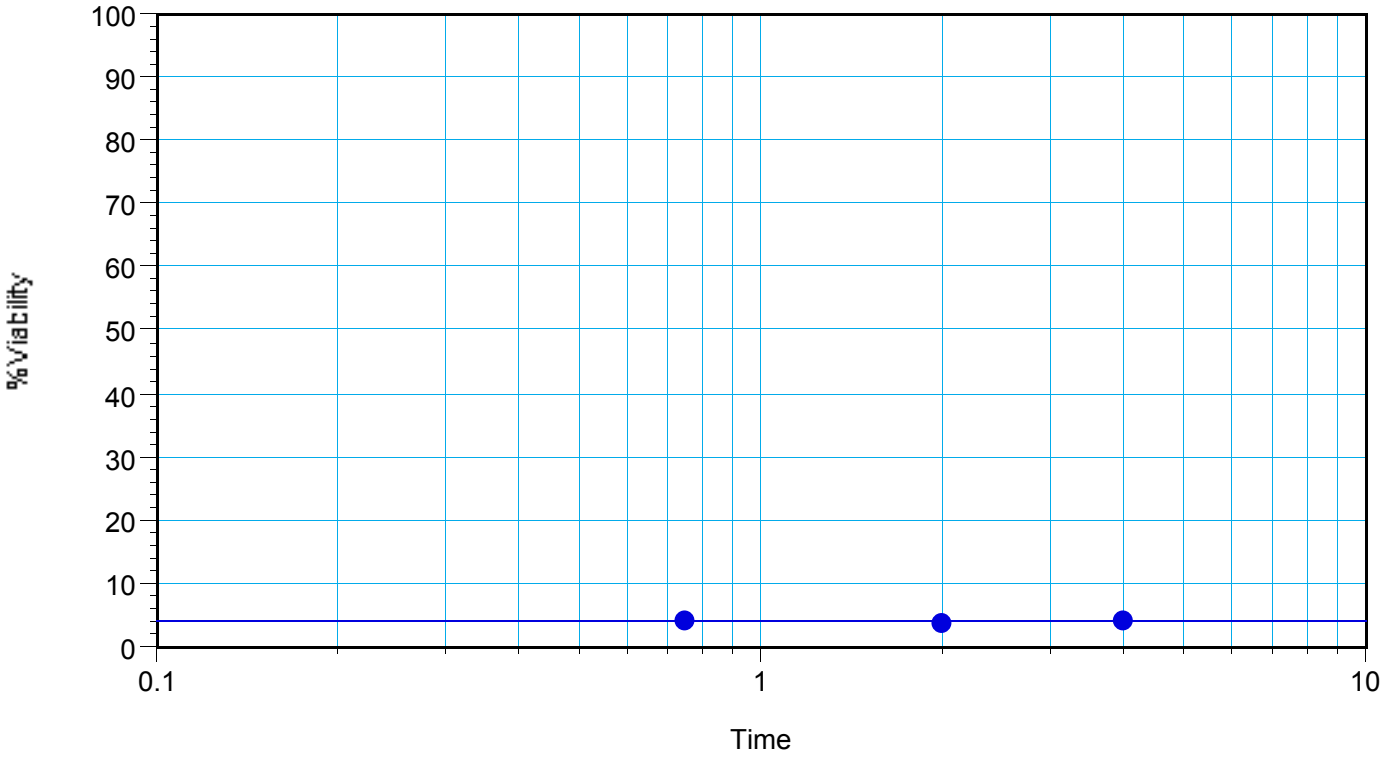
	y = A + B * Log(x):	A	B	R^2
●	Plot#1 (Product1: Time vs %Viability)	96.114	-65.437	0.976

Product2Graph



y = A + B * Log(x):	A	B	R^2
Plot#1 (Product2: Time vs %Viability)	19.71	-14.672	0.784

PositiveControlGraph



	y = A + B * Log(x):	A	B	R^2
●	Plot#1 (PositiveControl: Time vs %Viability)	3.988	-0.178	0.129

Protocol #170118-407 Epi-Vaginal Tissues Test Date: 03/04/17

MTT ET- 50 in hours - Product 1 = 34.19

MTT ET- 50 in hours - Product2 = 6.06

MTT ET- 50 in hours - Positive Control = 1.09

Product #1 = Test Product : Broad Spectrum Hygiene Management (lot # 16180-1)
Product #2 = Reference Product : Dyna-Hex 2 (lot # 1021-912)
Positive Control = 1% Triton X-100 Soution Lot # 072016SBA exp 07-20-17
Negative Control = Sterile Water for Irrigation

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.039	0.038	0.925	0.334	0.103					1.276	1.511	1.456
B												
C												
D												
E		1.670	1.618	0.470	1.389	0.785	0.121					
F		1.770	1.479	1.102	1.993	1.057	0.140					
G		1.750	1.640	0.887	1.447	1.012	0.151					
H												

Endpoint
Lm1 570
Automix: Off
Calibrate: On

Plate Last Read:
8:17 AM 3/4/2017

Wavelength Combination: !Lm1
Mean Temperature: 22.9
Data Mode: Absorbance

Product1 (Hours)

Sample	Wells	Time	Values	MeanValue	%Viability	CV%
01	E2	1	1.670	1.730	122.30	3.06
	F2		1.770			
	G2		1.750			
02	E3	4	1.618	1.579	111.63	5.54
	F3		1.479			
	G3		1.640			
03	E4	18	0.470	0.820	57.95	39.22
	F4		1.102			
	G4		0.887			

Product2 (hours)

Sample	Wells	Time	Values	MeanValue	%Viability	CV%
01	E5	1	1.389	1.610	113.82	20.71
	F5		1.993			
	G5		1.447			
02	E6	4	0.785	0.951	67.23	15.34
	F6		1.057			
	G6		1.012			
03	E7	18	0.121	0.137	9.71	10.86
	F7		0.140			
	G7		0.151			

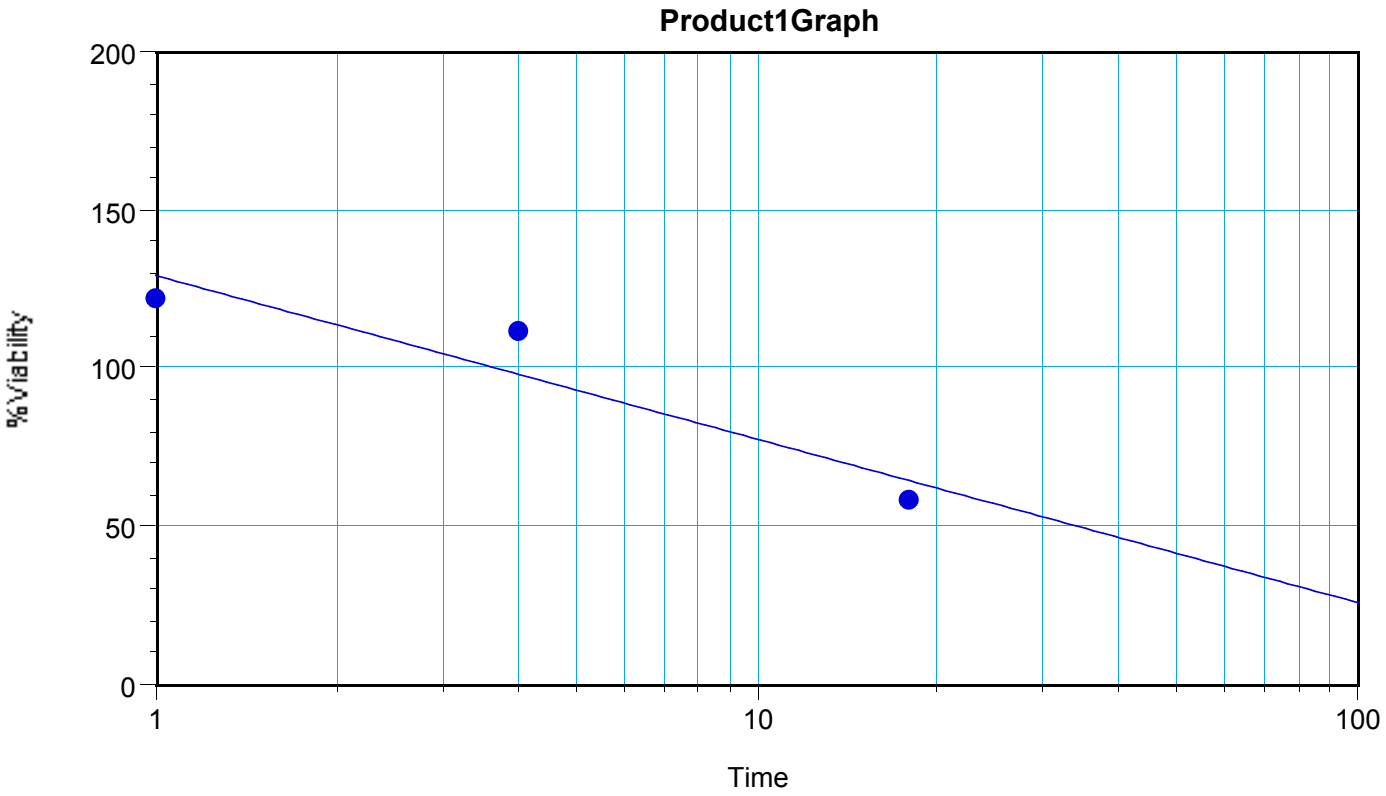
PositiveControl (Hours)

Sample	Wells	Time	Values	%Viability
01	A3	1	0.925	65.41
02	A4	2	0.334	23.64
03	A5	4	0.103	7.25

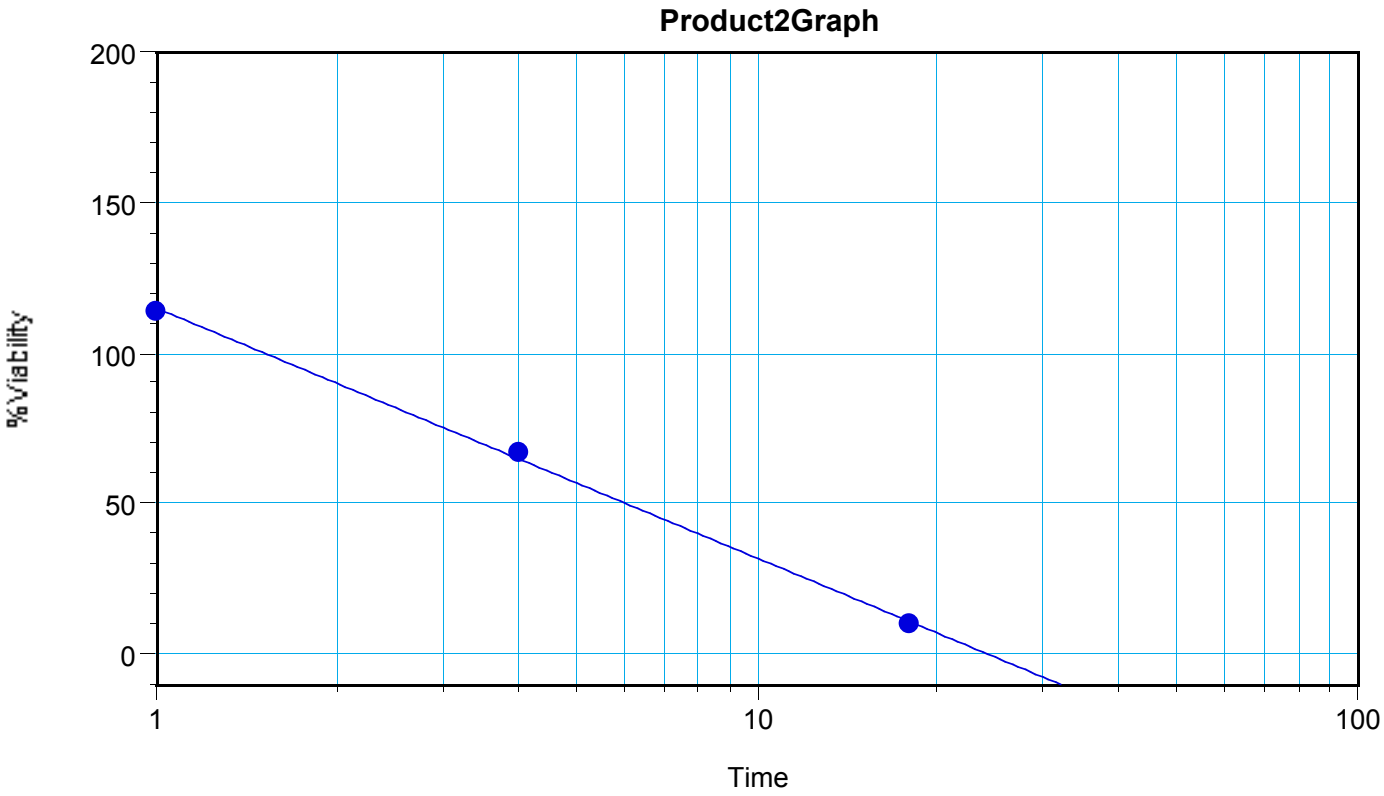
NegativeControl (Hours)

Sample	Wells	Time	Value
01	A10	4	1.276
	A11		1.511
	A12		1.456

Negative Control Value = 1.414

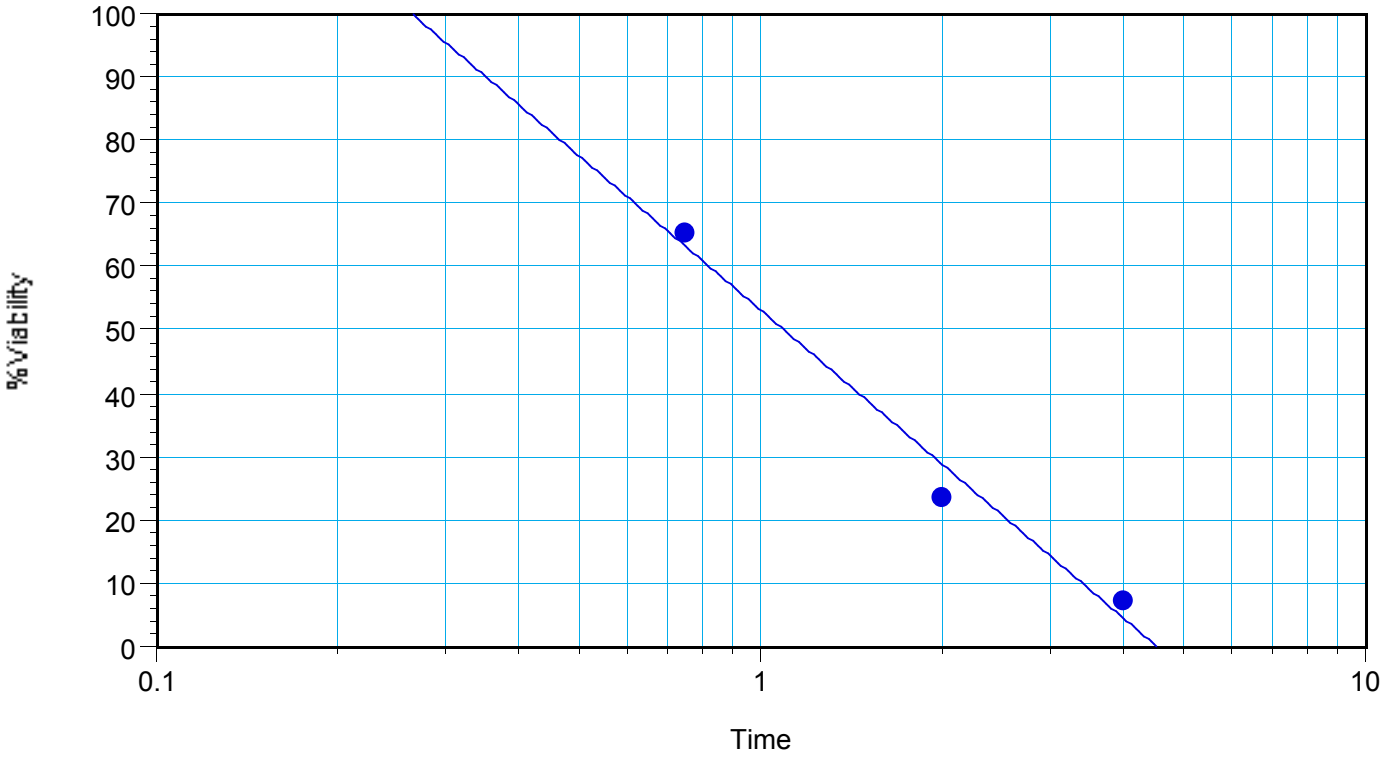


	y = A + B * Log(x):	A	B	R^2
●	Plot#1 (Product1: Time vs %Viability)	129.3	-51.698	0.886



y = A + B * Log(x):	A	B	R^2
Plot#1 (Product2: Time vs %Viability)	114.981	-83.013	0.999

PositiveControlGraph



	y = A + B * Log(x):	A	B	R^2
●	Plot#1 (PositiveControl: Time vs %Viability)	53.159	-81.192	0.978

Protocol #170118-407 Epi-Gingival Tissues Test Date: 03/09/17

MTT ET- 50 in hours - Product 1 = 399.36

MTT ET- 50 in hours - Product2 = 8.86

MTT ET- 50 in hours - Positive Control = 0.00

Product #1 = Test Product : Broad Spectrum Hygiene Management (lot # 16180-1)
Product #2 = Reference Product : Dyna-Hex 2 (lot # 1021-912)
Positive Control = 1% Triton X-100 Soution Lot # 102016BBA exp 10-20-17
Negative Control = Sterile Water for Irrigation

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.039	0.038	1.461	1.411	1.582					1.448	1.422	1.545
B												
C												
D												
E		1.618	1.542	1.251	1.674	1.535	0.132					
F		1.818	1.684	1.338	1.836	1.651	0.129					
G		1.906	1.639	1.203	1.800	1.627	0.133					
H												

Endpoint
Lm1 570
Automix: Off
Calibrate: On

Plate Last Read:
8:52 AM 3/10/2017

Wavelength Combination: !Lm1
Mean Temperature: 20.8
Data Mode: Absorbance

Product1 (Hours)

Sample	Wells	Time	Values	MeanValue	%Viability	CV%
01	E2	1	1.618	1.781	121.02	8.28
	F2		1.818			
	G2		1.906			
02	E3	4	1.542	1.622	110.20	4.48
	F3		1.684			
	G3		1.639			
03	E4	18	1.251	1.264	85.90	5.41
	F4		1.338			
	G4		1.203			

Product2 (hours)

Sample	Wells	Time	Values	MeanValue	%Viability	CV%
01	E5	1	1.674	1.770	120.28	4.79
	F5		1.836			
	G5		1.800			
02	E6	4	1.535	1.605	109.04	3.83
	F6		1.651			
	G6		1.627			
03	E7	18	0.132	0.131	8.92	1.36
	F7		0.129			
	G7		0.133			

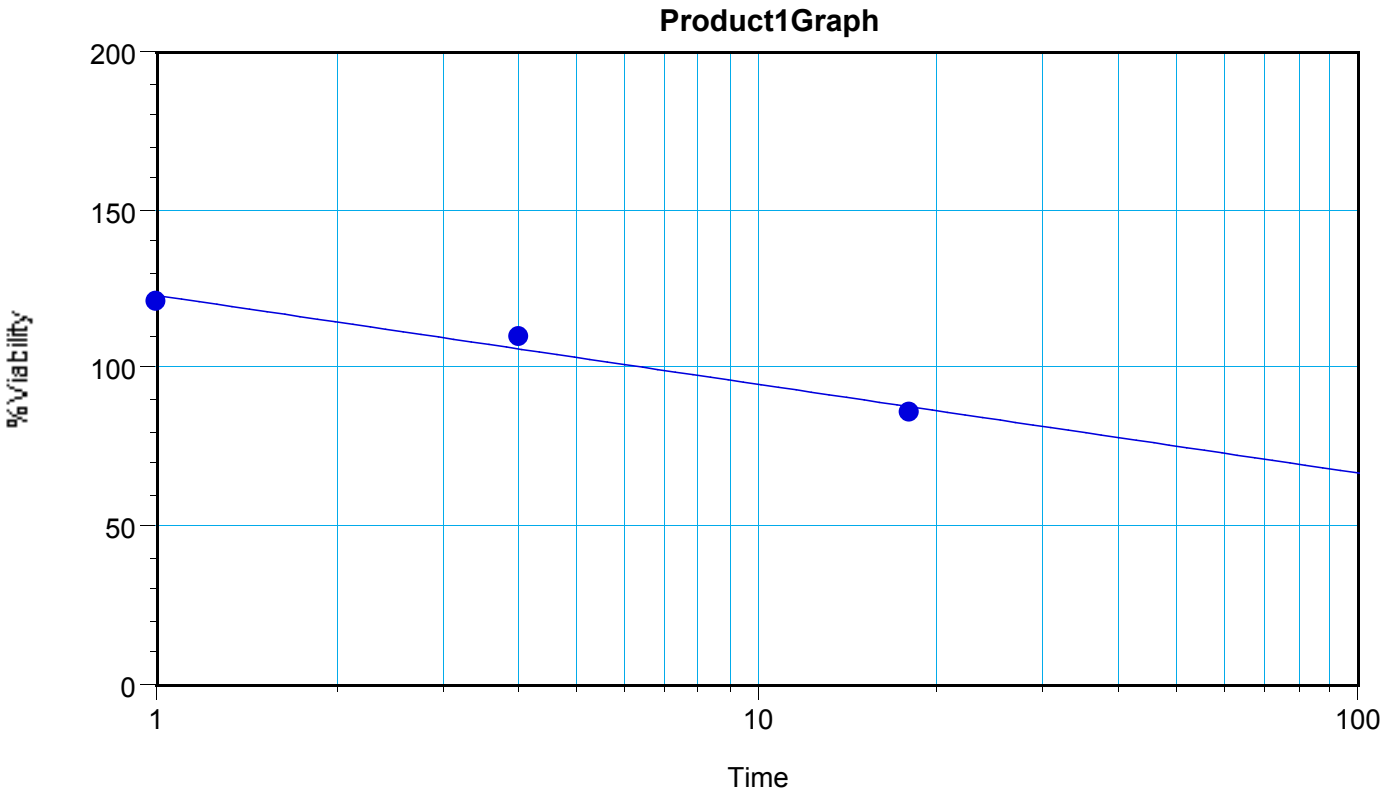
PositiveControl (Hours)

Sample	Wells	Time	Values	%Viability
01	A3	1	1.461	99.26
02	A4	2	1.411	95.89
03	A5	4	1.582	107.51

NegativeControl (Hours)

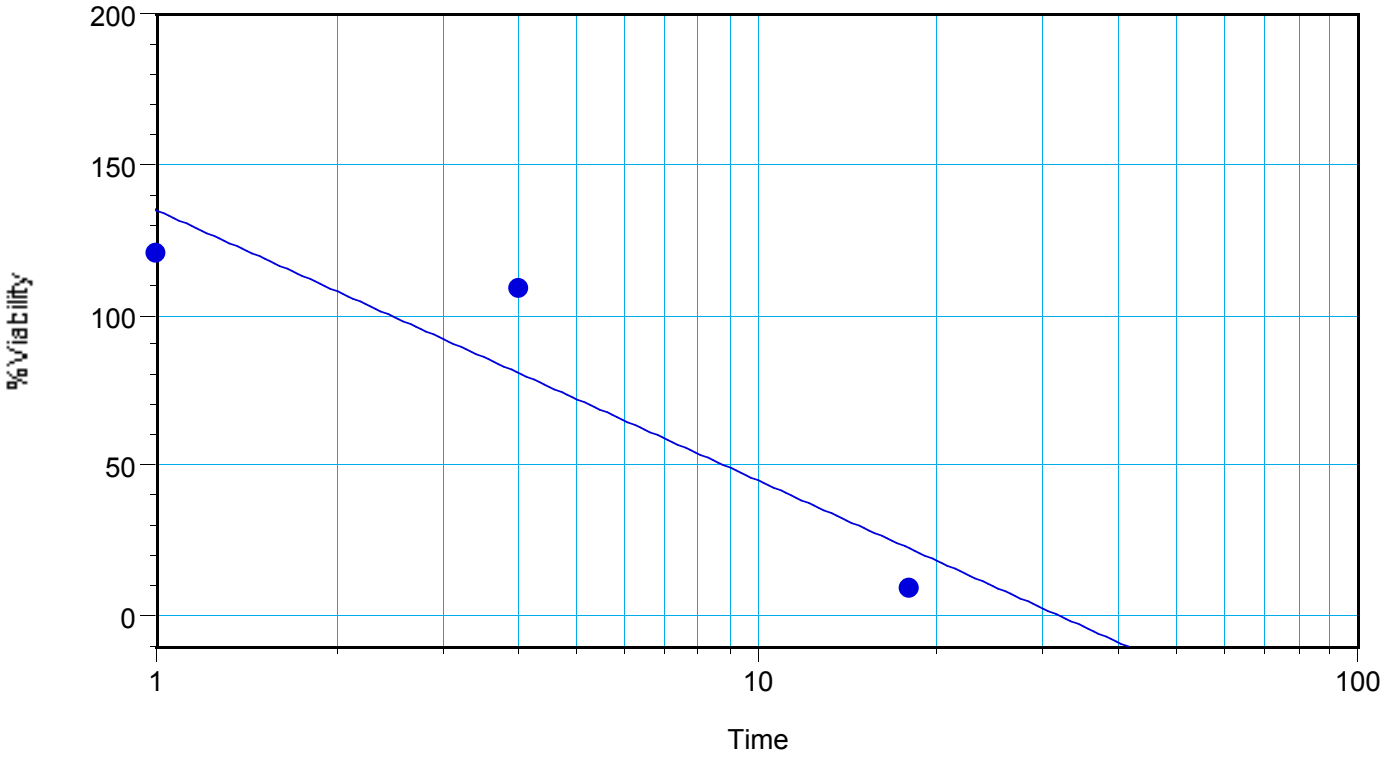
Sample	Wells	Time	Value
01	A10	4	1.448
	A11		1.422
	A12		1.545

Negative Control Value = 1.472



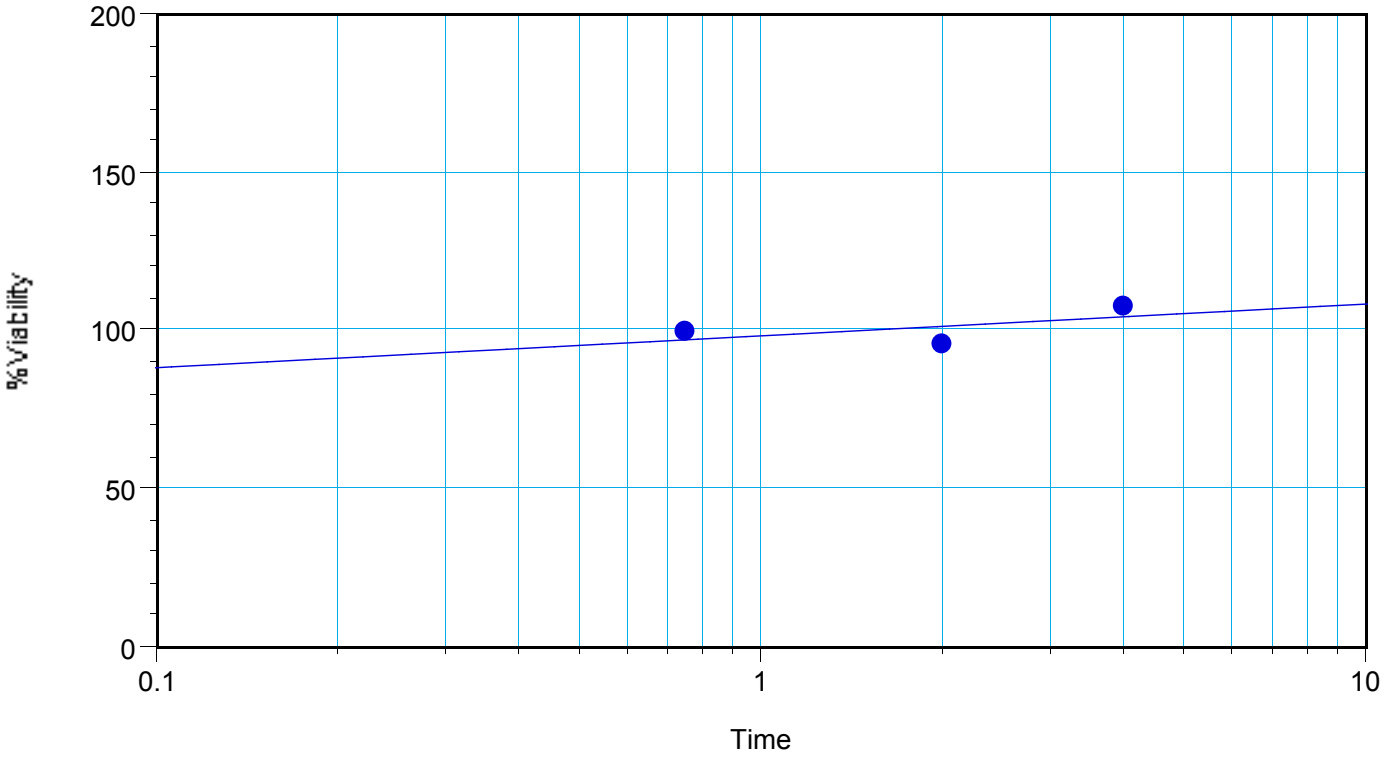
	y = A + B * Log(x):	A	B	R^2
●	Plot#1 (Product1: Time vs %Viability)	123.106	-28.103	0.963

Product2Graph



y = A + B * Log(x):	A	B	R^2
Plot#1 (Product2: Time vs %Viability)	134.904	-89.63	0.842

PositiveControlGraph



	y = A + B * Log(x):	A	B	R^2
●	Plot#1 (PositiveControl: Time vs %Viability)	98.277	10.066	0.378

Protocol #170118-407 Epi-Intestinal Tissues Test Date: 03/04/17

MTT ET- 50 in hours - Product 1 = 56.13

MTT ET- 50 in hours - Product2 = 1.42

MTT ET- 50 in hours - Positive Control = 0.73

Product #1 = Test Product : Broad Spectrum Hygiene Management (lot # 16180-1)
Product #2 = Reference Product : Dyna-Hex 2 (lot # 1021-912)
Positive Control = 0.3% Triton X-100 Soution Lot # 102816ZSA exp 10-28-17
Negative Control = Sterile Water for Irrigation

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.039	0.038	0.467	0.164	0.083					0.847	0.975	0.886
B												
C												
D												
E		0.783	0.902	0.556	0.594	0.138	0.128					
F		0.836	0.715	0.464	0.530	0.155	0.106					
G		0.778	0.681	0.563	0.619	0.138	0.103					
H												

Endpoint
Lm1 570
Automix: Off
Calibrate: On

Plate Last Read:
8:27 AM 3/4/2017

Wavelength Combination: !Lm1
Mean Temperature: 23.2
Data Mode: Absorbance

Product1 (Hours)

Sample	Wells	Time	Values	MeanValue	%Viability	CV%
01	E2	1	0.783	0.799	88.53	4.02
	F2		0.836			
	G2		0.778			
02	E3	4	0.902	0.766	84.88	15.56
	F3		0.715			
	G3		0.681			
03	E4	18	0.556	0.528	58.49	10.54
	F4		0.464			
	G4		0.563			

Product2 (hours)

Sample	Wells	Time	Values	MeanValue	%Viability	CV%
01	E5	1	0.594	0.581	64.39	7.87
	F5		0.530			
	G5		0.619			
02	E6	4	0.138	0.144	15.92	6.86
	F6		0.155			
	G6		0.138			
03	E7	18	0.128	0.112	12.46	12.24
	F7		0.106			
	G7		0.103			

PositiveControl (Hours)

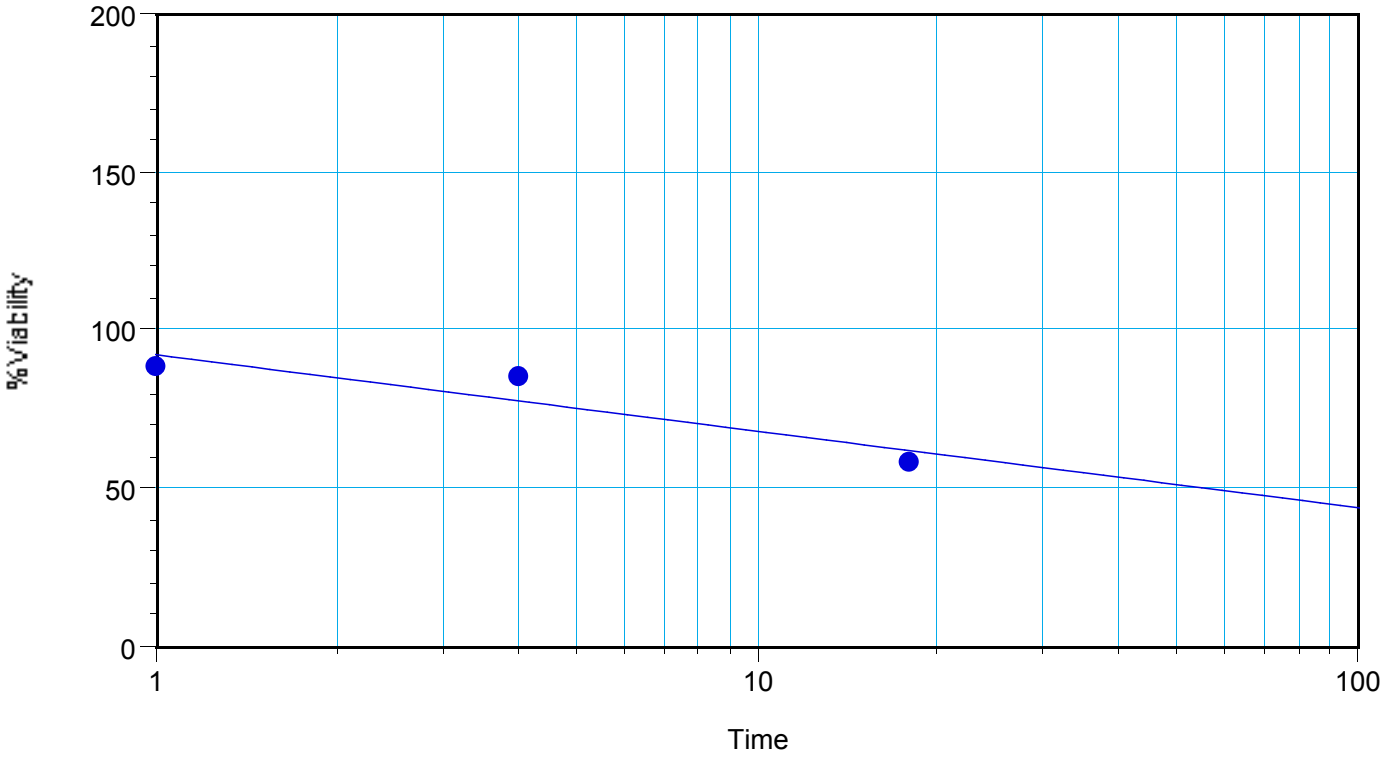
Sample	Wells	Time	Values	%Viability
01	A3	1	0.467	51.71
02	A4	2	0.164	18.14
03	A5	4	0.083	9.14

NegativeControl (Hours)

Sample	Wells	Time	Value
01	A10	4	0.847
	A11		0.975
	A12		0.886

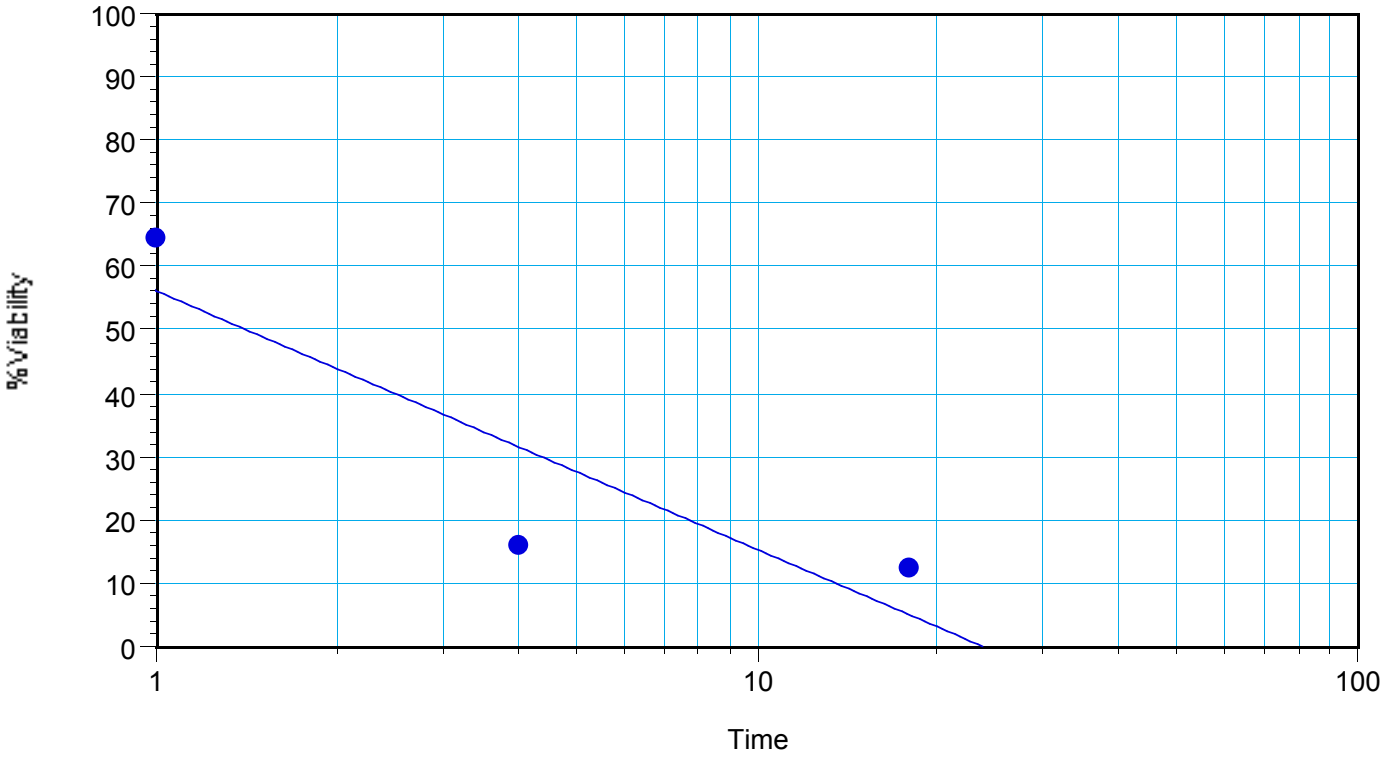
Negative Control Value = 0.902

Product1Graph



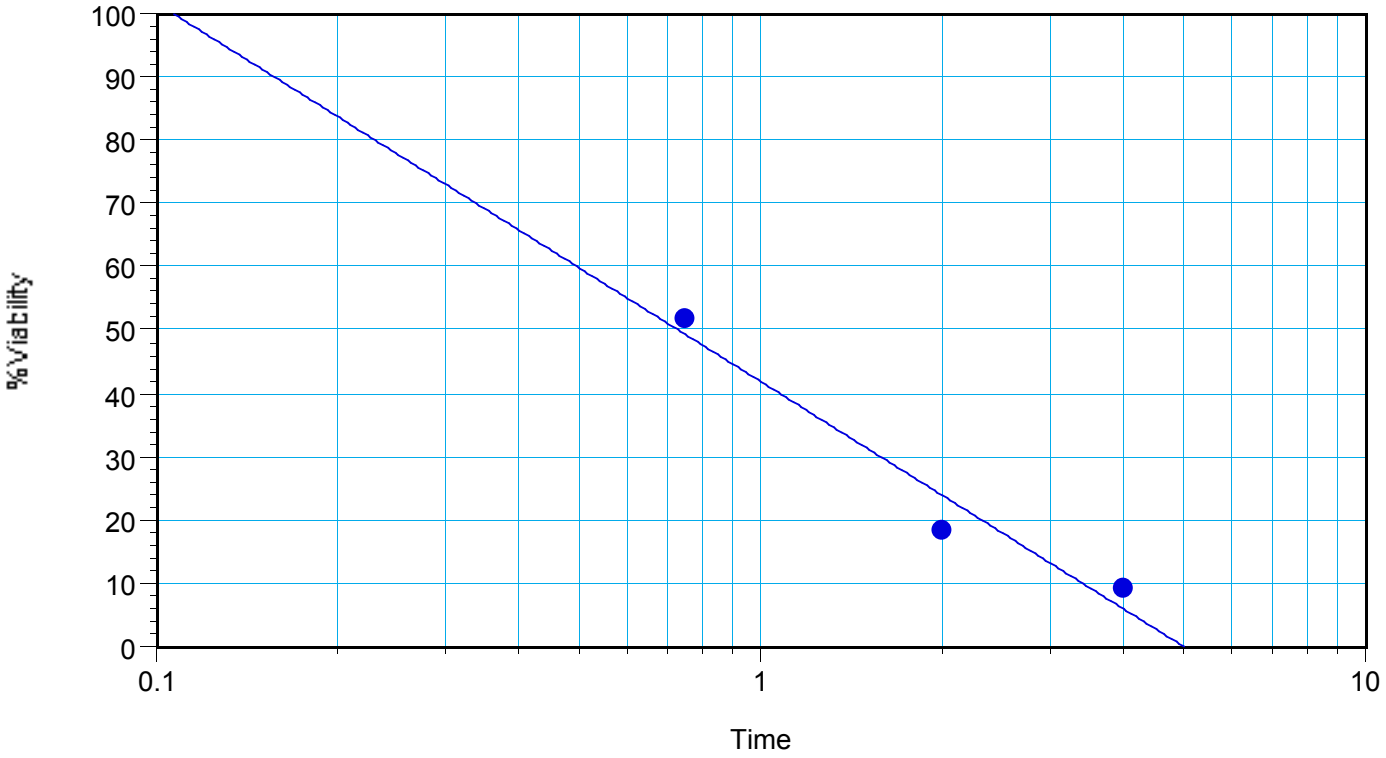
y = A + B * Log(x):	A	B	R^2
Plot#1 (Product1: Time vs %Viability)	92.257	-24.157	0.856

Product2Graph



	y = A + B * Log(x):	A	B	R^2
●	Plot#1 (Product2: Time vs %Viability)	56.217	-40.86	0.781

PositiveControlGraph



	y = A + B * Log(x):	A	B	R^2
●	Plot#1 (PositiveControl: Time vs %Viability)	41.867	-59.896	0.951