

*Nm-2 (Batch No.: 101), Nm-44 (Batch No.: 102), Nm-22 (Batch No.: 103), Nm-5 (Batch No.: 104), Nm-10 (Batch No.: 105), Nm-20 (Batch No.: 106), Nm-6 (Batch No.: 107), Nm-9 (Batch No.: 108), Nm-7 (Batch No.: 109), Nm-3 (Batch No.: 110), Nm-33 (Batch No.: 111), Nm-4 (Batch No.: 112)*

**SCREENING OF 12 COMPOUNDS VIABILITY AND PROLIFERATION EFFECTS ON SK-ES-1, KATO III and MPanc-96 CELL LINES**

Study No: IDL/007/CTX

*Final Technical Report*

Submitted to:  
Idealiza Ltd.

**December 29, 2009**

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**DATA PAGE**

Protocol Signed by the Study Director:	December 16, 2009
Protocol Signed by the Study Director:	December 17, 2009
Initiation of Study:	December 20, 2009
Completion of Study:	December 24, 2009
HBI Study No.:	IDL/008/CTX
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Sponsor:	Idealiza Ltd. Israel
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Study Director:	_____
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	Date

Total No. of Pages: **13**

**1. OBJECTIVE**

The objective of this study was to assess the effect of 10 Test Items on the viability and proliferation of 4 different cell lines using the Alamar Blue detector.

**2. TEST MATERIALS**

**2.1 Test Items:**

Test Item No.	Test Item	Physical State	Batch Number	Molecular Weigh/ Concentration	Supplied By	Storage Conditions
1	Nm-2	Powder	101	150.92	Idealiza	18-28°C
2	Nm-44	Powder	102	696.67	Idealiza	18-28°C
3	Nm-22	Powder	103	369.94	Idealiza	18-28°C
4	Nm-5	Powder	104	318.87	Idealiza	18-28°C
5	Nm-10	Powder	105	259.7	Idealiza	18-28°C
6	Nm-20	Powder	106	616.5	Idealiza	18-28°C
7	Nm-6	Powder	107	305.83	Idealiza	18-28°C
8	Nm-9	Powder	108	544.52	Idealiza	18-28°C
9	Nm-7	Powder	109	319.85	Idealiza	18-28°C
10	Nm-3	Powder	110	612.64	Idealiza	18-28°C
11	Nm-33	Powder	111	927.0	Idealiza	18-28°C
12	Nm-4	Powder	112	290.32	Idealiza	18-28°C

**2.2 Positive Control:**

Name:	<b>Doxorubicin</b>
Lot No.:	08G01PB
Physical state:	Liquid
Supplied by:	TEVA Medical
Concentration:	2 mg/ml
Storage conditions:	2-8°C
Sterility:	Non-Sterile
Expiry Date:	July 2011

**2.3 Adjunct Materials:**

2.3.1 Name:	AlamarBlue™
Catalog no.	BUF012B
Batch No.:	061008C
Physical state:	Liquid
Manufactured by:	Serotec
Storage conditions:	2-8°C
Expiry Date:	February 1, 2011

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2.3.2	Name:	DMSO
	Catalog No.:	D-5879
	Batch No.:	078K0692
	Supplied by:	Sigma
	Physical state:	Liquid
	Storage conditions:	18-28°C
	Expiry Date:	August 2010

## 2.4 Culture Growth Medium:

2.4.1	Name:	<b>SK-ES-1 Growth Medium</b>
	Content:	McCoy's 5a Medium supplemented with 2mM L-Glutamine, 10% FBS (Fetal Bovine Serum), 100U/ml Penicillin and 100µg/ml Streptomycin
	Batch No.:	101209
	Components supplied by:	Biological Industries, Israel
	Storage conditions:	2-8°C
	Expiry Date:	
2.4.2	Name:	<b>KATO III Growth Medium</b>
	Content:	Iscove's Medium supplemented with 4mM L-Glutamine, 20% FBS, 100U/ml Penicillin and 100µg/ml Streptomycin and 1.5g/L Sodium Bicarbonate
	Batch No.:	071209
	Components supplied by:	Biological Industries, Israel
	Storage conditions:	2-8°C
	Expiry Date:	
2.4.3	Name:	<b>MPanc-96 Growth Medium</b>
	Content:	RPMI supplemented with 2mM L-Glutamine, 10% FBS, 1mM Sodium Pyruvate, 100U/ml Penicillin and 100µg/ml Streptomycin, 1% Non Essential Amino Acid
	Batch No.:	071209
	Components supplied by:	Biological Industries, Israel
	Storage conditions:	2-8°C
	Expiry Date:	

## 2.5 Samples Preparation:

Test Items were dissolved in 100% DMSO to obtain 10mM final concentration. Stock solutions were diluted × 5000 with Growth Medium to achieve working solutions of 2µM. The Vehicle Control solution contained DMSO at a final concentration of 0.01% in Growth Medium.

## 2.6 Test System:

- SK-ES-1 Human sarcoma cell line, ATCC # HTB-86
- KATO III Human gastric carcinoma cell line, ATCC # HTB-103
- MPanc-96 (AsPC-1) Human pancreatic adenocarcinoma cell line, ATCC # CRL-2380 (CRL-1682)

## 2.7 Test Procedure:

Cells were thawed and passed at least once. Exponentially growing cultures of each cell line were collected, centrifuged, counted and seeded at the density of 5000cells/100µl/ well in pre-warmed Growth Medium in 5 rows of 96-well tissue culture plates each.

Plate 1 with one row of each cell line was seeded to serve as Time 0 (T<sub>0</sub>). Plates 2-5 were used for the Test Items, 2 plates for each time point (24 and 48 hours).

The Plates were incubated until the next day at 37±1°C, humidified, 5±0.5% CO<sub>2</sub>/air, to enable cells adherence to the well.

At the following day the following procedures were performed:

**Plate 1 - T<sub>0</sub> plate:** 100µl of fresh Growth Medium were added followed by the addition of 22µl AlamarBlue™. The fluorescent signal (Excitation 544nm/Emission 590nm) was measured following 4 and 24 hours incubation time.

**Plates 2-5 - Treatment plates:** 100µl of the Test Items were added to the cells according to the following plate plans.

**Plates 6-7 – Blank:** 100µl of the Test Items (4 wells for each Test Item) were added to the wells in the respective Growth Medium of each cell line. Plates 6-7 serves as fluorescent signal blank for the Test Item (no cells were seeded).

### Plates Plan:

#### Plate 1 – T<sub>0</sub>

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	<b>SK-ES-1</b>											
<b>B</b>	<b>KATO III</b>											
<b>C</b>	<b>MPanc-96</b>											

Alamar Blue was added and signal was measured after 4 and 24 hours thereafter.

#### Plates 2 and 4

	1	2	3	4	5	6	7	8	9	10	11	12	
<b>A</b>	Test Item 1			Test Item 2			Test Item 3			<b>SK-ES-1</b>			
<b>B</b>	Test Item 4			Test Item 5			Test Item 6						
<b>C</b>	Test Item 7			Test Item 8			Test Item 9						
<b>D</b>	Test Item 10			Test Item 11			Test Item 12						
<b>E</b>	Positive Control			Vehicle Control									
<b>F</b>	Test Item 1			Test Item 2			Test Item 3			<b>KATO III</b>			
<b>G</b>	Test Item 4			Test Item 5			Test Item 6						
<b>H</b>	Test Item 7			Test Item 8			Test Item 9						

#### Plate 3 and 5

	1	2	3	4	5	6	7	8	9	10	11	12	
<b>A</b>	Test Item 10			Test Item 11			Test Item 12			<b>KATO III</b>			
<b>B</b>	Positive Control			Vehicle Control									
<b>C</b>	Test Item 1			Test Item 2			Test Item 3			<b>MPanc-96</b>			
<b>D</b>	Test Item 4			Test Item 5			Test Item 6						
<b>E</b>	Test Item 7			Test Item 8			Test Item 9						
<b>F</b>	Test Item 10			Test Item 11			Test Item 12						
<b>G</b>	Positive Control			Vehicle Control									
<b>H</b>													

Plates were incubated with the Test Materials for 24 hours (Plates 2 and 3) and 48 hours (Plates 4 and 5) at 37±1°C, humidified 5±0.5% CO<sub>2</sub>/air, after which Alamar Blue was added. The signal was measured 4 and 24 hours thereafter.

**Plate 6**

	1	2	3	4	5	6	7	8	9	10	11	12	
<b>A</b>	Test Item 1			Test Item 2			Test Item 3			<b>SK-ES-1 Growth Medium</b>			
<b>B</b>	Test Item 4			Test Item 5			Test Item 6						
<b>C</b>	Test Item 7			Test Item 8			Test Item 9						
<b>D</b>	Test Item 10			Test Item 11			Test Item 12						
<b>E</b>	Positive Control			Vehicle Control									
<b>F</b>	Test Item 1			Test Item 2			Test Item 3			<b>KATO III Growth Medium</b>			
<b>G</b>	Test Item 4			Test Item 5			Test Item 6						
<b>H</b>	Test Item 7			Test Item 8			Test Item 9						

**Plate7**

	1	2	3	4	5	6	7	8	9	10	11	12	
<b>A</b>	Test Item 10			Test Item 11			Test Item 12			<b>KATO III Growth Medium</b>			
<b>B</b>	Positive Control			Vehicle Control									
<b>C</b>	Test Item 1			Test Item 2			Test Item 3			<b>MPanc-96 Growth Medium</b>			
<b>D</b>	Test Item 4			Test Item 5			Test Item 6						
<b>E</b>	Test Item 7			Test Item 8			Test Item 9						
<b>F</b>	Test Item 10			Test Item 11			Test Item 12						
<b>G</b>	Positive Control			Vehicle Control									
<b>H</b>													

Plates were incubated with the Test Materials for 24 hours at 37±1°C, humidified 5±0.5% CO<sub>2</sub>/air, after which Alamar Blue was added. The signal was measured 4 and 24 hours thereafter.

**3. DATA EVALUATION**

3.1 Signals from blank wells (plates 6-7) are averaged for each Test Item and subtracted from the Cells + Test Items (Plates 2-5) fluorescence signal values.

3.2 Average fluorescence signals are calculated for each 4 replicate wells.

3.3 Cell growth is expressed as percent of Vehicle Control:

$$\frac{(\text{Averaged O.D Test Item} - \text{Average O.D } T_0)}{(\text{Averaged O.D Vehicle Control} - \text{Average O.D } T_0)} \times 100$$

3.4 Results interpretation:

- Negative value (<0%) indicates cytotoxic effect
- Positive value (0-100%) indicates cytostatic effect.
- Value >100 indicates proliferative effect.

#### 4. **RESULTS:**

**Table 1: The effect of 12 Test Items on the proliferation of 3 different cell lines.**

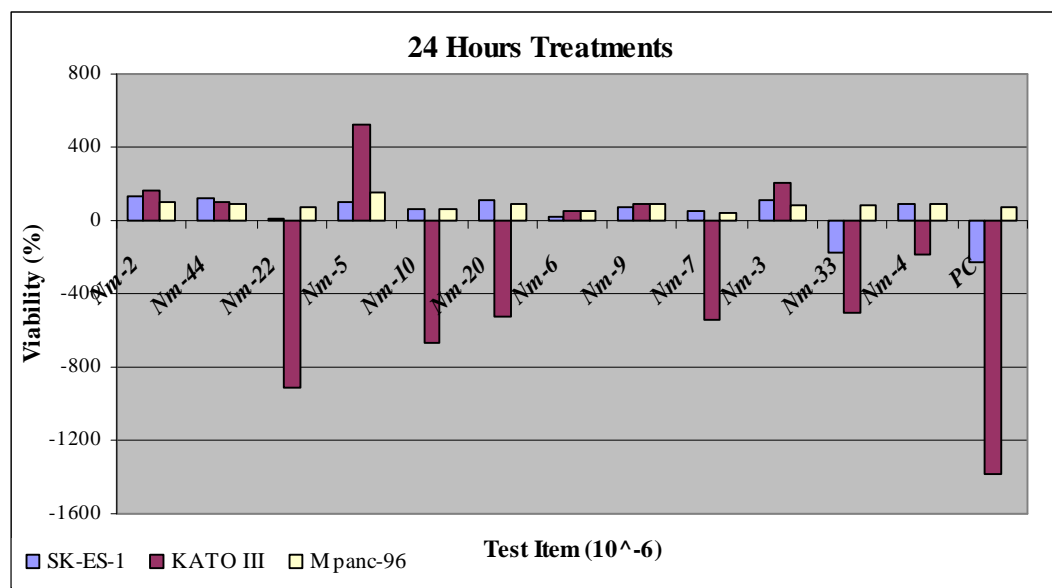
SK-ES-1, KATO III and MPanc-96 cells were treated with 12 Test Items ( $10^{-6}$ M), Doxorubicin ( $10^{-6}$ M) as Positive Control, and Growth Medium + 0.02% DMSO as Vehicle Control for a period of 24 and 48 hours, after which they were subjected to the AlamarBlue™ assay. The fluorescent signal (O.D.) at excitation/emission of 544nm/590nm was measured following 24 hrs of incubation with AlamarBlue™. Mean values were calculated and after subtraction of  $T_0$  mean values results were expressed as % of Vehicle Control. Raw data is presented in the Appendix. The cytotoxic effect (↓) proliferative effect (↑) and cytostatic effect (=) are pointed by symbols.

Cell Line	Test Item	Percent of Vehicle Control (%)				General Trend
		24 hr		48 hr		
		%	Effect	%	Effect	
SK-ES-1	Nm-2	136	↑	118	↑	Proliferative
	Nm-44	125	↑	110	↑	Proliferative
	Nm-22	10	=	41	=	Cytostatic
	Nm-5	98	=	98	=	Cytostatic
	Nm-10	66	=	64	=	Cytostatic
	Nm-20	108	↑	107	↑	Proliferative
	Nm-6	16	=	37	=	Cytostatic
	Nm-9	72	=	94	=	Cytostatic
	Nm-7	50	=	60	=	Cytostatic
	Nm-3	118	↑	103	↑	Proliferative
	Nm-33	-170	↓	-72	↓	Cytotoxic
	Nm-4	93	=	100	=	Cytostatic
Doxorubicin	-223	↓	-77	↓	Cytotoxic	
KATO III	Nm-2	169	↑	218	↑	Proliferative
	Nm-44	102	↑	107	↑	Proliferative
	Nm-22	-916	↓	-451	↓	Cytotoxic
	Nm-5	525	↑	318	↑	Proliferative
	Nm-10	-670	↓	-321	↓	Cytotoxic
	Nm-20	-527	↓	-63	↓	Cytotoxic
	Nm-6	51	=	61	=	Cytostatic
	Nm-9	93	=	14	=	Cytostatic
	Nm-7	-544	↓	-223	↓	Cytotoxic
	Nm-3	208	↑	93	=	Proliferate / Cytostatic
	Nm-33	-504	↓	-402	↓	Cytotoxic
	Nm-4	-182	↓	108	↑	Cytotoxic / Proliferate
Doxorubicin	-1381	↓	-1048	↓	Cytotoxic	

Cell Line	Test Item	Percent of Vehicle Control (%)				General trend
		24 hr		48 hr		
		%	Effect	%	Effect	
MPanc 96	Nm-2	105	↑	123	↑	Proliferative
	Nm-44	92	=	94	=	Cytostatic
	Nm-22	71	=	106	↑	Cytostatic / Proliferate
	Nm-5	153	↑	179	↑	Proliferative
	Nm-10	57	=	73	=	Cytostatic
	Nm-20	97	=	89	=	Cytostatic
	Nm-6	47	=	78	=	Cytostatic
	Nm-9	88	=	109	↑	Cytostatic / Proliferate
	Nm-7	37	=	63	=	Cytostatic
	Nm-3	85	=	119	↑	Cytostatic / Proliferate
	Nm-33	87	=	-63	↓	Cytostatic / Cytotoxic
	Nm-4	91	=	96	=	Cytostatic
	Doxorubicin	74	=	24	=	Cytostatic

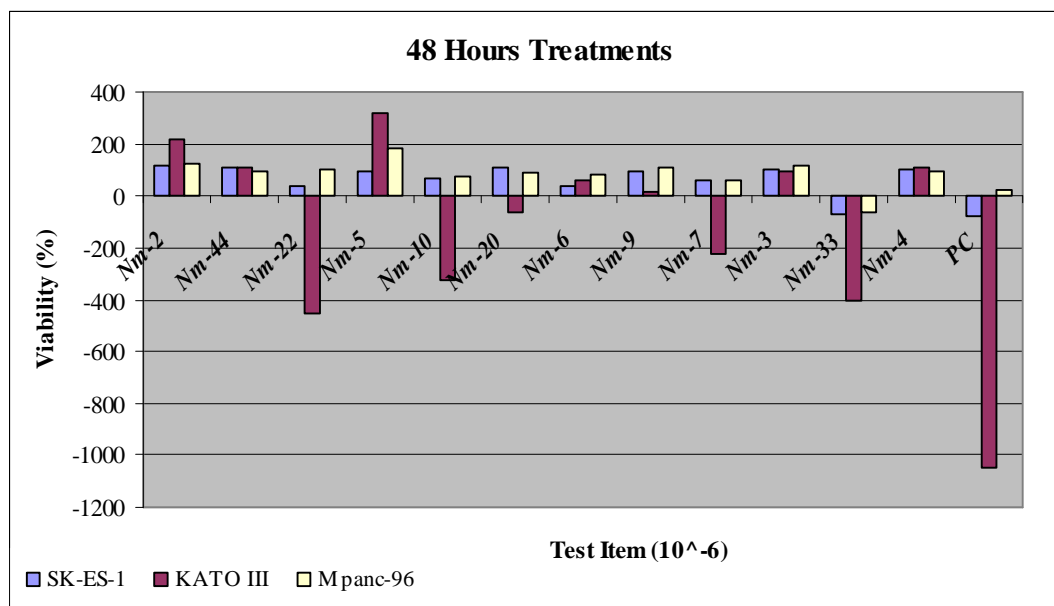
**Figure 1**

The effect of Test Items as percent of Vehicle Control (VC) 24 hours post treatments.



**Figure 2**

The effect of Test Items as percent of Vehicle Control (VC) 48 hours post treatments.



## 5. **CONCLUSIONS:**

The effect of the 12 Test Items on the proliferation of the 3 different cell lines was assessed using the Alamar Blue™ detector.

The different Test Items showed various levels of cytotoxic, cytostatic and proliferative effects, on the different cancer cell lines (SK-ES-1, KATO III and MPanc-96) relative to the Vehicle Control.

The analysis was performed on the reading values following 24 hours with Alamar Blue™ since the signals were higher than after 4 hours with Alamar Blue. The reading values following 4 hours with Alamar Blue™ are available in the Raw Data (Appendix #1).

Following 24 hours of Test Items exposure at a concentration of 10<sup>-6</sup>M, cytotoxic effect was observed on SK-ES-1 cell line only by Nm-33. On KATO III cytotoxic effect was caused by Nm-22, Nm-10, Nm-20, Nm-7, Nm-33 and Nm-4. On MPanc-96 cell line a cytotoxic effect wasn't observed. The Positive Control (Doxorubicin 10<sup>-6</sup>M) caused a cytotoxic effect on both SK-ES-1 and KATO III. As for MPanc-96, a cytostatic effect was shown after exposure to the Positive Control (Table 1 and Figure 1).

Following 48 hours of Test Items exposure, the cytotoxic effect on SK-ES-1 was the same as after 24 hours exposure. On KATO III a cytotoxic effect was shown in Nm-22, Nm-10, Nm-20, Nm-7 and Nm-33. On MPanc-96 a cytotoxic effect was shown only after treatment with Nm-33 (Table 1 and Figure 2).

## **APPENDIX 1- INDIVIDUAL DATA**

### **Fluorescent signals (Excitation 544nm/Emission 590nm)**

#### **Plate 1 – T<sub>0</sub>: Cell lines and Growth Medium (4hr Alamar-Blue™)**

	1	2	3	4	5	6	7	8	9	10	11	12
SK-ES-1	12.08	11.87	11.56	11.54	11.60	11.40	11.23	11.25	11.64	11.79	11.28	11.84
KATO III	14.29	13.78	13.49	13.52	12.76	13.41	12.78	13.52	12.71	12.74	13.41	14.05
MPanc 96	21.22	20.00	21.16	20.13	17.76	19.12	18.37	17.61	16.75	18.45	17.53	18.85

#### **Plate 1 – T<sub>0</sub>: Cell lines and Growth Medium (24hr Alamar-Blue™)**

	1	2	3	4	5	6	7	8	9	10	11	12
SK-ES-1	102.7	97.16	93.99	94.41	91.57	88.43	91.81	92.65	92.35	91.29	90.98	93.93
KATO III	211.4	195.9	195.9	199.8	206.3	206.1	205.9	208.4	199.2	202.7	198.3	195.6
MPanc 96	183.9	181.2	183.9	178.2	179.6	181.5	185.7	180.3	175.2	181.6	179.4	186.3

#### **Cell Lines and Test Item, 24 hours exposure (4hr Alamar Blue™)**

##### **Plate 2**

	1	2	3	4	5	6	7	8	9	10	11	12
SK-ES-1	17.17	14.92	15.29	17.10	15.55	16.59	14.96	14.25	14.45	13.62	13.75	14.35
	15.55	15.43	14.39	14.18	14.77	15.25	14.83	13.64	15.29	15.88	13.21	15.88
	13.60	12.51	13.41	13.24	13.67	13.84	13.67	13.90	13.47	14.02	12.67	15.11
	16.52	15.42	14.68	13.49	11.48	10.91	11.25	10.78	13.89	14.33	13.00	15.15
	11.42	10.45	10.91	10.93	17.67	15.78	15.01	15.11				
KATO III	16.18	14.56	14.47	14.09	16.45	14.23	16.23	12.89	12.74	13.18	17.24	13.27
	16.51	14.71	15.46	16.27	13.77	12.02	16.13	12.50	21.03	13.10	12.82	13.85
	17.51	15.92	16.86	14.59	17.35	12.71	16.30	14.63	17.89	13.67	14.31	16.33

##### **Plate 3**

	Nm-2	Nm-44	Nm-22	Nm-5	Nm-10	Nm-20	Nm-6	Nm-9	Nm-7	Nm-3	Nm-33	Nm-4
KATO III	19.08	17.36	17.19	16.31	15.33	13.57	14.02	13.80	15.42	15.44	16.89	16.17
	17.22	12.54	13.91	13.24	15.42	12.44	17.24	13.61				
MPanc 96	36.58	29.96	26.99	26.18	31.61	25.77	25.47	25.66	23.40	24.62	28.47	31.18
	42.66	28.63	25.81	28.43	22.75	22.45	20.85	21.87	31.05	26.54	34.44	36.99
	34.66	23.96	23.31	22.89	24.60	22.45	20.78	23.16	24.00	21.85	24.40	26.05
	39.91	27.20	27.16	27.02	24.20	23.53	22.07	22.80	28.66	24.76	28.90	36.18
	31.41	25.10	25.02	22.25	28.02	22.85	26.08	28.34				

#### **Cell Lines and Test Item, 48 hours exposure (4hr Alamar Blue™)**

##### **Plate 4**

	1	2	3	4	5	6	7	8	9	10	11	12
SK-ES-1	18.93	15.11	14.91	17.44	15.91	15.64	14.43	15.39	13.97	13.93	13.67	15.96
	14.79	13.60	12.65	13.84	13.56	15.30	12.27	12.93	16.67	14.26	13.97	23.10
	12.45	12.19	11.88	11.81	15.15	18.72	12.85	13.84	12.42	13.17	12.81	12.91
	20.00	16.04	15.27	15.29	10.13	10.19	10.35	10.21	17.31	15.33	15.85	16.74
	9.859	9.761	9.924	9.697	19.17	19.94	13.58	14.38				
KATO III	17.64	14.23	13.70	15.97	14.01	15.89	12.46	14.55	11.55	13.17	11.31	10.69
	15.77	14.82	14.43	13.28	13.96	12.39	14.00	12.07	15.06	17.00	14.41	15.24
	19.37	12.96	15.90	12.23	15.32	15.04	13.32	13.47	14.67	13.29	12.05	14.63

**Plate 5**

	Nm-2	Nm-44	Nm-22	Nm-5	Nm-10	Nm-20	Nm-6	Nm-9	Nm-7	Nm-3	Nm-33	Nm-4
KATO III	13.28	12.57	12.81	11.89	11.46	11.11	10.76	11.04	12.80	13.50	13.15	22.05
	10.38	11.28	10.95	10.21	12.15	11.82	18.71	14.04				
MPanc 96	40.06	31.16	34.94	26.19	31.16	25.86	22.87	32.54	22.74	22.37	22.14	27.63
	32.91	24.50	31.10	23.34	19.08	17.65	16.13	19.15	22.92	23.36	22.32	39.27
	26.08	25.05	26.74	20.96	24.23	22.17	27.12	17.63	18.90	23.38	26.09	25.48
	29.20	34.22	28.84	28.97	14.45	15.68	18.94	15.40	27.95	27.44	25.84	29.60
	20.91	20.72	20.55	16.94	24.02	20.50	27.26	25.39				

**Cell Lines and Test Item, 24 hours exposure (24hr Alamar Blue™)**

**Plate 2**

	1	2	3	4	5	6	7	8	9	10	11	12
SK-ES-1	169.5	154.7	162.2	165.2	161.0	164.7	154.7	152.6	116.8	112.2	112.3	114.8
	155.5	145.1	145.3	147.3	140.9	135.8	132.5	133.6	154.9	153.5	146.6	151.3
	121.6	110.1	114.4	119.6	139.1	138.2	134.7	139.6	129.3	130.5	124.9	132.6
	167.8	152.3	154.1	149.2	47.06	43.79	44.26	42.16	148.5	148.5	143.4	144.3
	26.04	24.74	24.33	24.26	154.3	150.3	147.7	143.2				
KATO III	238.3	220.8	222.4	226.9	235.7	227.1	220.1	213.8	187.0	193.5	184.1	176.1
	244.3	235.7	240.5	243.1	205.8	196.3	198.5	178.5	224.8	196.6	202.1	201.3
	230.5	211.6	220.9	207.6	236.8	214.2	228.3	217.8	210.0	185.6	192.2	210.5

**Plate 3**

	Nm-2	Nm-44	Nm-22	Nm-5	Nm-10	Nm-20	Nm-6	Nm-9	Nm-7	Nm-3	Nm-33	Nm-4
KATO III	243.4	223.5	221.9	224.6	209.0	209.8	200.2	184.0	215.5	210.6	208.8	218.3
	190.8	158.9	161.3	160.2	229.4	224.7	232.2	210.1				
MPanc 96	279.4	267.9	257.5	253.9	260.0	257.9	248.0	258.3	249.5	235.4	237.9	246.8
	317.3	306.3	291.8	291.4	240.8	238.4	226.0	227.8	263.3	247.8	260.6	263.2
	250.8	224.9	230.7	225.1	255.6	251.2	249.1	255.4	223.8	215.1	214.3	226.3
	264.0	248.8	250.1	244.2	258.2	247.2	251.4	253.7	259.8	246.4	251.0	264.5
	252.5	239.7	247.0	240.2	264.0	261.0	258.6	261.6				

**Cell Lines and Test Item, 48 hours exposure (24hr Alamar Blue™)**

**Plate 4**

	1	2	3	4	5	6	7	8	9	10	11	12
SK-ES-1	270.1	248.2	250.1	243.7	245.1	248.1	238.3	236.9	159.8	155.4	161.8	160.1
	246.7	214.8	224.5	226.2	195.9	190.4	180.5	182.3	238.1	229.4	233.0	254.5
	162.2	157.4	149.2	151.2	232.3	230.2	209.2	220.4	187.8	189.1	172.6	176.8
	251.7	235.5	224.8	227.8	24.21	23.46	21.98	23.07	233.1	231.9	227.8	229.0
	18.85	18.31	18.17	18.24	242.0	239.3	221.7	219.7				
KATO III	279.8	232.4	229.6	234.5	232.8	231.8	230.4	232.1	181.3	178.9	170.5	156.1
	271.6	252.6	250.4	245.6	193.9	191.0	193.2	165.6	220.7	211.3	205.6	216.3
	234.4	223.8	234.5	209.8	235.4	222.1	222.6	208.6	203.7	192.3	181.9	207.8

**Plate 5**

	Nm-2	Nm-44	Nm-22	Nm-5	Nm-10	Nm-20	Nm-6	Nm-9	Nm-7	Nm-3	Nm-33	Nm-4
KATO III	241.6	225.8	236.9	217.2	180.4	168.3	176.0	182.4	229.3	223.9	222.8	251.8
	104.7	106.1	108.4	112.5	238.2	226.8	236.0	223.1				
MPanc 96	304.8	286.6	285.3	275.5	275.3	263.2	259.1	267.8	266.7	280.8	274.5	280.5
	343.1	320.7	332.6	326.9	251.0	241.5	246.0	265.2	254.0	265.3	249.8	278.9
	257.5	253.5	251.8	255.4	276.6	283.3	281.3	269.4	230.9	251.5	245.7	243.6
	290.3	284.4	278.7	286.8	129.8	146.5	154.9	163.7	266.7	269.7	254.5	279.9
	221.6	233.8	217.5	184.4	264.1	270.4	272.5	276.8				

**Growth Medium and Test Item, 24 hours exposure (4hr Alamar Blue™)**

**Plate 6**

	1	2	3	4	5	6	7	8	9	10	11	12
SK-ES-1	10.06	9.923	9.789	9.825	9.674	9.690	9.663	9.646	9.825	9.877	9.692	9.798
	10.03	9.710	9.791	9.558	9.795	9.651	9.592	9.663	9.488	9.578	9.451	9.448
	9.617	9.925	9.885	10.18	10.21	10.17	10.09	10.13	9.291	9.554	9.845	9.439
	9.608	9.577	10.15	9.767	9.769	9.577	9.670	9.608	9.714	9.555	9.424	9.432
	10.10	10.06	9.855	8.972	9.587	9.543	9.713	9.494				
KATO III	8.821	8.573	8.528	8.492	8.460	8.358	8.364	8.314	8.427	8.626	8.296	8.237
	8.659	8.390	8.424	8.401	8.419	8.308	8.356	8.365	8.341	8.309	8.240	8.120
	8.634	8.367	8.294	5.531	8.492	8.437	8.364	8.440	8.224	8.251	7.920	8.272

**Plate 7**

	Nm-2	Nm-44	Nm-22	Nm-5	Nm-10	Nm-20	Nm-6	Nm-9	Nm-7	Nm-3	Nm-33	Nm-4
KATO III	8.718	8.570	8.536	8.620	8.750	8.751	8.628	8.678	8.792	8.745	8.769	8.403
	9.101	8.498	8.513	8.555	8.572	8.368	8.676	8.346				
MPanc 96	10.33	10.07	8.105	10.56	10.28	10.06	10.32	10.06	10.19	10.21	10.11	9.932
	10.21	9.929	8.488	10.10	10.18	10.55	9.911	10.51	10.02	9.972	9.721	10.38
	9.998	10.38	10.60	10.19	10.75	9.932	10.54	10.05	10.17	10.11	10.15	10.67
	10.53	10.56	10.62	10.40	10.80	10.34	10.09	9.998	10.12	10.36	10.03	9.958
	10.42	10.08	10.20	10.11	10.12	10.37	10.04	10.05				

**Growth Medium and Test Item, 24 hours exposure (24hr Alamar Blue™)**

**Plate 6**

	1	2	3	4	5	6	7	8	9	10	11	12
SK-ES-1	17.07	16.75	17.02	16.58	16.37	16.60	16.36	16.61	16.48	17.06	16.61	16.94
	16.81	16.60	16.65	16.93	16.47	16.79	16.74	16.51	16.12	16.43	16.09	16.29
	17.36	16.97	16.86	16.52	16.52	16.62	16.30	16.64	16.38	16.70	16.23	16.50
	17.55	17.00	16.43	16.61	16.40	16.86	16.19	16.51	16.57	16.50	16.59	16.58
	17.90	17.48	17.72	17.59	16.92	16.90	16.48	16.77				
KATO III	18.73	18.53	18.32	18.40	18.07	18.25	18.08	18.14	18.20	18.46	18.14	18.14
	19.14	18.41	18.45	18.42	18.36	18.34	18.44	18.35	18.18	18.37	17.85	18.06
	18.43	18.23	17.92	13.06	18.56	18.57	18.42	18.60	18.37	18.22	18.18	18.63

**Plate 7**

	Nm-2	Nm-44	Nm-22	Nm-5	Nm-10	Nm-20	Nm-6	Nm-9	Nm-7	Nm-3	Nm-33	Nm-4
KATO III	18.68	18.27	18.02	17.99	18.02	18.06	17.84	18.08	17.98	18.18	18.11	18.36
	18.85	18.74	18.68	18.58	18.30	18.12	17.92	18.15				
MPanc 96	14.84	14.64	14.18	14.89	14.55	14.79	14.65	14.51	14.35	14.64	14.75	14.52
	14.73	14.57	15.24	14.56	14.33	14.41	14.34	14.43	14.13	14.17	14.14	14.24
	14.92	14.53	14.31	14.43	14.08	14.34	14.10	14.16	14.51	14.48	14.61	14.47
	14.89	14.57	14.44	14.49	14.39	14.52	14.38	14.51	14.34	14.39	14.32	14.36
	15.25	15.19	15.12	15.12	14.50	14.57	14.43	14.48				