

*Ng-3 (K103), Ng-4 (B119), Ng-5 (B116), Ng-6 (B117), Ng-7 (K202), Ng-10 (K105),  
Ng-22 (B105), OF-2 (B118), OF-3 (B112), OF-7(B101)*

**THE EFFECT OF 10 COMPOUNDS ON THE VIABILITY AND PROLIFERATION  
OF HT-29, DU-145, PC-3 AND SK-OV-3 - SCREENING ASSAY**

Study No: IDL/007/CTX

*Final Technical Report*

Submitted to:  
Idealiza Ltd.

September 15, 2009

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**DATA PAGE**

Protocol Signed by the Study Director:	August 23, 2009
Initiation of Study:	August 23, 2009
Completion of Study:	August 28, 2009
HBI Study No.:	IDL/007/CTX
Archive Location:	Harlan Biotech Israel Kiryat Weizmann, Bldg. 13B Rehovot 76326, Israel
Sponsor:	Idealiza Ltd. Israel
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Testing Facility:	Harlan Biotech Israel Kiryat Weizmann, Bldg. 13B Rehovot 76326, Israel

Study Director:

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\_\_\_\_\_  
Date

**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	Expiry Date
1	Ng-3	Powder	K103	612.84	Idealiza	RT	NA
2	Ng-4	Powder	B119	290.32	Idealiza	RT	NA
3	Ng-5	Powder	B116	318.87	Idealiza	RT	NA
4	Ng-6	Powder	B117	305.83	Idealiza	RT	NA
5	Ng-7	Powder	K202	319.86	Idealiza	RT	NA
6	Ng-10	Powder	K105	578.37	Idealiza	RT	NA
7	Ng-22	Powder	B105	369.94	Idealiza	RT	NA
8	OF-2	Powder	B118	407.99	Idealiza	RT	NA
9	OF-3	Powder	B112	927.0	Idealiza	RT	NA
10	OF-7	Powder	B101	479.02	Idealiza	RT	NA

**2.2 Reference Item:**

Name:	<b>Doxorubicin</b>
Catalog No.:	LO1DB
Lot. No.:	713474
Physical state:	Liquid
Supplied by:	EBEWE Pharm
Concentration:	2 mg/ml
Storage conditions:	2-8°C
Sterility:	Non-Sterile
Expiry Date:	November 2011

**2.3 Adjunct Materials:**

2.3.1 Name:	AlamarBlue™
Catalog no.	BUF012B
Batch No.:	030908B
Physical state:	Liquid
Manufactured by:	Serotec
Storage conditions:	2-8°C
Expiry Date:	December, 11 2009

2.3.2 Name:	DMSO
Catalog No.:	D-5879
Supplied by:	Sigma
Physical state:	Liquid
Storage conditions:	Room temperature 18-28°C

## 2.4 Culture Growth Medium:

- |       |  |  |
|-------|--|--|
| 2.4.2 | Name:<br>Content:                              | <b>DU-145 Growth Medium</b><br>EMEM supplemented with 10 FBS (Fetal Bovine Serum), 2mM glutamine, 1 non-essential amino acid, 1mM Na pyruvate, 1.5gr/lit Sodium bicarbonate, 100U/ml penicillin and 100µg/ml streptomycin. |
|       | Components supplied by:<br>Storage conditions: | Biological Industries, Israel<br>2-8°C   |
| 2.4.3 | Name:<br>Content:                              | <b>PC-3 Growth Medium</b><br>RPMI-1640 medium, supplemented with 10 FBS (Fetal Bovine Serum), 1mM Sodium pyruvate, 2mM L-glutamine, 1 non-essential amino acid, 100U/ml penicillin and 100µg/ml streptomycin.              |
|       | Components supplied by:<br>Storage conditions: | Biological Industries, Israel<br>2-8°C   |
| 2.4.4 | Name:<br>Content:                              | <b>HT-29 and SK-OV-3 Growth Medium</b><br>DMEM medium, supplemented with 10 FBS (Fetal Bovine Serum), 1mM Sodium pyruvate, 2mM L-glutamine, 1 non-essential amino acid, 100U/ml penicillin and 100µg/ml streptomycin.      |
|       | Components supplied by:<br>Storage conditions: | Biological Industries, Israel<br>2-8°C   |

## 2.5 Samples Preparation:

Test Items were dissolved in 100 DMSO to obtain a final concentration of 10mM. Stock solutions were diluted ×5000 with Growth Medium to achieve working solutions of 0.2µM. Vehicle Control solution contains 0.01 DMSO in Growth Medium.

## 2.6 Test System:

Four different cell lines were used in this test:

HT-29 – Colon adenocarcinoma cell line, ATCC # HTB-38

DU-145 – Human prostate carcinoma, ATCC # HTB-81

PC-3 – Human prostate adenocarcinoma, ATCC # CRL-1435

SK-OV-3 – Human ovarian adenocarcinoma, ATCC # HTB-77

## 2.7 Test Procedure:

Cells were thawed and passed at least once. Exponentially growing cultures of each cell line were harvested, centrifuged, counted and seeded at the density of 5000 cells/100µl/ well in pre-warmed Growth Medium in 4 rows of 96-well tissue culture plates each. For the 4 cell lines 2 plates were seeded (two cell lines in each plate, see plate template below) for each time point (24, 48 and 72 hours) generating a total of 6 plates. An additional plate (plate 3) with one row of each cell line was seeded to serve as Time 0 (T<sub>0</sub>). The Plates were incubated until the next day at 37±1°C, 5±0.5 CO<sub>2</sub> and 95±5 humidity, to enable cells adherence to the well.

At the following day the following procedures were performed:

**Plate 3 - 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 an appropriate incubation time (4 hours and 24 hours).

**Plates 1-2 – treatment plates:** 100µl of the various treatment solutions of Test Items were added to the cells according to the following plate plan.

Plate 1-2 – Test Items:

	1	2	3	4	5	6	7	8	9	10	11	12	
A													
B	TI-1	TI-2	TI-3	TI-4	TI-5	TI-6	TI-7	TI-8	TI-9	TI-10	RI	VC	1 <sup>st</sup> Cell Line
C													
D													
E													
F	TI-1	TI-2	TI-3	TI-4	TI-5	TI-6	TI-7	TI-8	TI-9	TI-10	RI	VC	2 <sup>nd</sup> Cell Line
G													
H													

TI – Test Item RI – Reference Item VC – Vehicle Control

**Plate 4 – Blank:** In an additional plate, individual wells were filled with 100µL/well Growth Medium and 100µL/well of the different treatments (4 wells each). This plate serves as fluorescent signal blank.

Plates were incubated with Test Items for 24, 48 and 72 hours. Following the above incubation period, 22µL/well of AlamarBlue™ was added to plates 1-2 and plate 4 and the fluorescent signal (Excitation 544nm/Emission 590nm) was measured following an appropriate incubation time (4 hours and 24 hours).

### 3. DATA EVALUATION

- 3.1 Signals from blank wells (plate 4) were averaged for each treatment and subtracted from the treatments fluorescence signal values.
- 3.2 Average fluorescence signals were calculated for each set of replicate wells.
- 3.3 Cell growth was expressed as percent of Vehicle Control:

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

Negative value <0 indicates cytotoxic effect

Positive value 0-100 indicates cytostatic effect while >100 indicates proliferative effect.

#### 4. RESULTS:

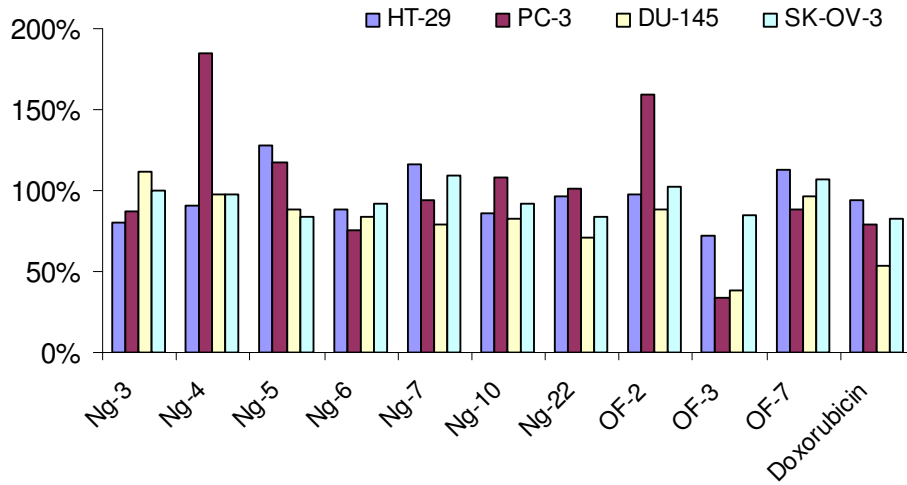
**Table 1** The effect of 10 Test Items on the proliferation of 4 different cell lines.

DU-145, PC-3, SK-OV-3 and HT-29 cells were treated with 10 Test Items (10 $\mu$ M), Doxorubicin (1 $\mu$ M) as Positive Control (P.C.), and Vehicle (Medium +0.02 DMSO) as Negative Control (N.C.) for a period of 24, 48, and 72hours, after which they were subjected to the AlamarBlue™ assay. The fluorescent signal (O.D.) at excitation/emission of 544nm/590nm was measured following 4 hrs of incubation with AlamarBlue™. Mean values were calculated and after subtraction of t<sub>0</sub> 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	Conc.	Percent of Vehicle Control (%)						General trend
			24 hr		48 hr		72 hr		
			%	Effect	%	Effect	%	Effect	
DU-145	Ng-3	10 <sup>-7</sup> M	111	↑	131	↑	77	=	Prolif/Cytostatic
	Ng-4		98	=	101	↑	68	=	Cytostatic
	Ng-5		89	=	100	=	36	=	Cytostatic
	Ng-6		84	=	105	↑	80	=	Cytostatic
	Ng-7		80	=	105	↑	72	=	Cytostatic
	Ng-10		82	=	103	↑	55	=	Cytostatic
	Ng-22		71	=	86	=	84	=	Cytostatic
	OF-2		88	=	112	↑	80	=	Cytostatic
	OF-3		38	=	21	=	9	=	Cytostatic
	OF-7		97	=	91	=	51	=	Cytostatic
	Doxorubicin	10 <sup>-6</sup> M	53	=	-12	↓	-13	↓	Cytotoxic
PC-3	Ng-3	10 <sup>-7</sup> M	87	=	663	↑	210	↑	Proliferative
	Ng-4		185	↑	268	↑	47	=	Prolif/Cytostatic
	Ng-5		118	↑	25	=	43	=	Cytostatic
	Ng-6		76	=	275	↑	-84	↓	Prolif/Cytotoxic
	Ng-7		94	=	348	↑	56	=	Prolif/Cytostatic
	Ng-10		108	↑	340	↑	40	=	Prolif/Cytostatic
	Ng-22		101	↑	332	↑	27	=	Prolif/Cytostatic
	OF-2		160	↑	389	↑	-56	↓	Prolif/Cytotoxic
	OF-3		34	=	124	↑	-51	↓	Prolif/Cytotoxic
	OF-7		89	=	308	↑	70	=	Prolif/Cytostatic
	Doxorubicin	10 <sup>-6</sup> M	79	=	46	=	-62	↓	Cytosta/Cytotoxic
SK-OV-3	Ng-3	10 <sup>-7</sup> M	100	=	108	↑	101	↑	Cytostatic/Prolif
	Ng-4		98	=	89	=	76	=	Cytostatic
	Ng-5		84	=	86	=	92	=	Cytostatic
	Ng-6		92	=	81	=	86	=	Cytostatic
	Ng-7		109	↑	72	=	82	=	Cytostatic
	Ng-10		92	=	93	=	83	=	Cytostatic
	Ng-22		84	=	79	=	75	=	Cytostatic
	OF-2		102	↑	95	=	95	=	Cytostatic
	OF-3		85	=	78	=	61	=	Cytostatic
	OF-7		107	↑	98	=	102	↑	Cytostatic
	Doxorubicin	10 <sup>-6</sup> M	83	=	37	=	13	=	Cytostatic
HT-29	Ng-3	10 <sup>-7</sup> M	80	=	95	=	103	↑	Cytostatic
	Ng-4		91	=	92	=	81	=	Cytostatic
	Ng-5		128	↑	118	↑	119	↑	Proliferative
	Ng-6		88	=	101	↑	95	=	Cytostatic
	Ng-7		116	↑	113	↑	82	=	Cytostatic
	Ng-10		86	=	89	=	83	=	Cytostatic
	Ng-22		96	=	91	=	84	=	Cytostatic
	OF-2		98	=	89	=	70	=	Cytostatic
	OF-3		72	=	50	=	22	=	Cytostatic
	OF-7		113	↑	94	=	94	=	Cytostatic
	Doxorubicin	10 <sup>-6</sup> M	94	=	57	=	18	=	Cytostatic

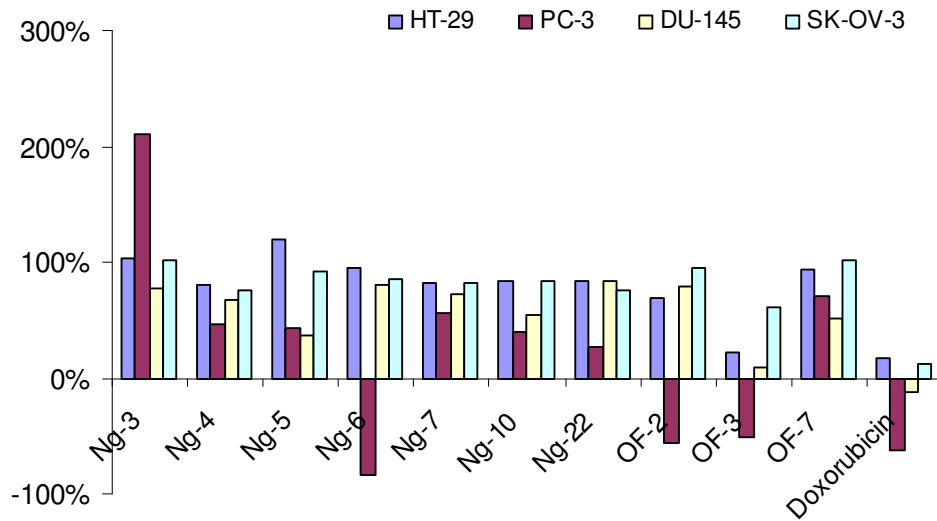
**Figure 1**

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) 72 hours post treatments.



**Table 2 The effect of 10 Test Items on the proliferation of 4 different cell lines.**

DU-145, PC-3, SK-OV-3 and HT-29 cells were treated with 10 Test Items (10 $\mu$ M), Doxorubicin (1 $\mu$ M) as Positive Control (P.C.), and Vehicle (Medium +0.02 DMSO) as Negative Control (N.C.) for a period of 24, 48, and 72hours, 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<sub>0</sub> 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	Conc.	Percent of Vehicle Control (%) in 96 hr	Effect
DU-145	Ng-3	10 <sup>-7</sup> M	111	↑
	Ng-4		106	↑
	Ng-5		-19	↓
	Ng-6		108	↑
	Ng-7		103	↑
	Ng-10		26	=
	Ng-22		104	↑
	OF-2		79	=
	OF-3		-13	↓
	OF-7		-11	↓
	Doxorubicin	10 <sup>-6</sup> M	-256	↓
PC-3	Ng-3	10 <sup>-7</sup> M	191	↑
	Ng-4		111	↑
	Ng-5		68	=
	Ng-6		12	=
	Ng-7		72	=
	Ng-10		77	=
	Ng-22		64	=
	OF-2		19	=
	OF-3		-37	↓
	OF-7		70	=
	Doxorubicin	10 <sup>-6</sup> M	-178	↓
SK-OV-3	Ng-3	10 <sup>-7</sup> M	101	↑
	Ng-4		105	↑
	Ng-5		107	↑
	Ng-6		109	↑
	Ng-7		104	↑
	Ng-10		108	↑
	Ng-22		97	=
	OF-2		104	↑
	OF-3		93	=
	OF-7		108	↑
	Doxorubicin	10 <sup>-6</sup> M	-39	↓
HT-29	Ng-3	10 <sup>-7</sup> M	98	=
	Ng-4		101	↑
	Ng-5		68	=
	Ng-6		102	↑
	Ng-7		103	↑
	Ng-10		50	=
	Ng-22		87	=
	OF-2		84	=
	OF-3		-30	↓
	OF-7		-17	↓
	Doxorubicin	10 <sup>-6</sup> M	30	=

## 5. CONCLUSIONS:

The effect of the 10 Test Items on the proliferation of the 4 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 (DU-145, PC-3 SK-OV-3 and HT-29) relative to the Vehicle Control.

In the first 24 hours following the addition of the Test Items in concentration of  $10^{-7}$ M neither of Test Items nor in the reference Item (Doxorubicin  $10^{-6}$ M) had shown a cytotoxic effect on the screened cell line (Table 1 and Figure 1).

Following 48 hours of Test Items exposure, cytotoxic effect was shown only in reference Item (Doxorubicin  $10^{-6}$ M) on DU-145 cells (Table 1).

After 72 hours, on PC-3 cells a clear cytotoxic effect was caused by Test Items Ng-6, OF-2 and OF-3 (final concentration of  $10^{-7}$ M) as well as in the Reference Item (Doxorubicin,  $10^{-6}$ M). The Reference Item continued to show cytotoxic effect on DU-145 cells (Table 1 and Figure 2).

The effect of Test Items on the cancer cell lines could be also shown 96 hours post treatments due to Alamar Blue™ measurements of 24 hours (Table 2) in addition to the 4 hours measurements (Table 1). The results displayed in Table 2 should be carefully considered since cells medium was not replaced during the 96 hours and cells may pass the logarithmic phase of growth, thus, it is reasonable that cells die due to high density or lack of nutrients and not affected by Test Items concentrations.

In consideration with the above the cytotoxic effect shown in Table 2 is as follows:

DU-145 cells in Test Items Ng-5, OF-3 and OF-7 in addition to Doxorubicin effect.

PC-3 cells only in OF-3 Test Item in addition to Doxorubicin.

SK-OV-3 cells only Doxorubicin had cytotoxic effect.

HT-29 cells OF-3 and OF-7 were cytotoxic.

**RAW DATA**

Plate 1 - Fluorescent signal (Excitation 544nm/Emission 590nm) 24 hours (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	VC	RI
DU-145	78.26	66.31	66.41	63.55	63.38	62.78	63.41	69.74	43.95	70.57	72.21	47.62
	65.29	53.70	54.74	55.70	53.90	53.38	47.25	54.04	37.77	62.60	57.57	43.10
	58.48	59.88	52.75	51.26	49.04	48.61	47.56	52.09	37.33	56.06	60.06	45.80
	54.30	56.16	53.69	49.28	46.87	48.04	43.75	49.05	37.23	50.76	51.58	43.96
PC-3	30.15	78.80	63.08	51.98	61.65	61.92	72.09	69.96	40.71	59.00	44.05	60.27
	34.78	27.30	30.29	28.52	29.28	26.84	27.46	27.77	28.50	22.59	35.78	25.23
	35.75	32.77	33.16	31.74	29.48	31.34	26.83	30.68	27.84	31.48	34.98	32.23
	45.39	45.65	33.74	32.51	32.30	34.31	31.80	45.82	33.14	39.23	39.94	32.64

Plate 1 - Fluorescent signal (Excitation 544nm/Emission 590nm) 48 hours (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
DU-145	89.73	66.41	72.85	76.28	72.97	70.23	67.76	78.66	36.33	66.96	64.61	22.34
	78.26	68.85	65.29	62.71	61.14	68.47	59.13	70.77	35.47	66.18	67.84	21.81
	76.07	59.83	67.61	74.68	72.09	66.29	59.92	74.68	32.61	59.14	68.30	21.55
	78.48	75.57	67.29	65.64	72.70	67.12	61.91	66.75	35.44	67.01	70.49	21.77
PC-3	78.06	58.89	27.45	63.56	63.42	64.16	65.91	70.73	49.06	64.08	27.76	52.62
	40.61	36.72	31.59	34.62	34.39	38.70	36.83	35.19	31.57	34.43	39.09	27.19
	33.29	26.65	28.31	25.80	28.01	27.53	28.50	27.86	23.68	28.04	30.90	24.61
	40.32	29.56	35.35	26.38	34.39	25.98	30.52	30.32	28.45	30.54	34.17	24.00

Plate 1 - Fluorescent signal (Excitation 544nm/Emission 590nm) 72 hours (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
DU-145	88.58	80.59	53.16	95.32	88.43	75.01	94.63	97.93	34.26	66.31	104.1	19.28
	78.76	61.82	51.38	80.09	77.24	72.23	82.42	79.46	32.27	59.13	88.63	18.97
	68.79	61.48	44.89	70.53	61.97	50.47	72.24	80.95	33.26	60.62	86.38	18.49
	67.08	74.22	51.59	66.21	63.72	44.12	73.78	52.22	28.43	55.50	84.23	18.44
PC-3	76.44	20.50	71.57	19.61	79.69	69.39	71.67	21.28	41.72	74.21	93.49	41.57
	22.82	74.82	19.33	19.34	19.33	19.73	19.47	20.36	17.54	20.20	20.57	17.92
	79.87	20.48	20.27	20.76	20.12	20.07	20.70	22.34	17.55	20.84	21.57	17.97
	28.00	25.43	27.37	22.89	25.58	25.45	23.52	31.31	19.46	36.98	28.05	18.76

Plate 1 - Fluorescent signal (Excitation 544nm/Emission 590nm) 24 hours (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
DU-145	560.2	554.8	534.9	544.2	548.0	543.3	541.5	556.8	391.6	543.1	548.1	175.7
	525.1	511.0	504.8	509.0	509.5	510.8	495.6	505.1	410.5	509.3	516.4	185.0
	516.2	516.2	485.8	484.6	478.3	480.3	472.5	485.6	382.1	485.5	502.2	189.4
	509.9	495.4	477.8	460.2	471.0	477.5	453.1	484.5	372.9	465.6	492.6	184.1
PC-3	279.4	413.6	397.8	386.7	385.7	394.9	394.3	419.8	373.8	414.6	322.9	391.7
	285.0	277.2	265.0	261.1	248.1	255.4	242.5	247.8	249.7	251.8	282.0	251.0
	293.0	287.0	280.1	274.8	267.5	279.6	253.6	277.7	252.0	264.5	288.4	249.9
	335.1	346.2	287.0	288.2	266.1	300.9	277.0	301.3	278.2	284.9	299.4	252.7

Plate 1 - Fluorescent signal (Excitation 544nm/Emission 590nm) 48 hours (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
DU-145	551.0	544.0	530.2	540.7	528.8	539.6	528.3	541.1	412.6	533.2	533.7	64.83
	535.6	528.7	513.1	522.4	513.8	522.2	520.2	526.5	395.7	518.5	526.4	62.34
	531.3	529.1	515.9	527.7	520.8	515.9	512.1	526.8	394.1	516.1	523.6	60.71
	536.7	531.1	508.8	512.1	517.2	510.9	497.5	512.4	402.5	507.9	521.9	61.30
PC-3	477.4	467.7	311.2	474.6	459.3	446.0	442.0	447.3	416.3	418.8	294.0	365.8
	340.0	330.7	301.6	309.3	300.8	306.5	295.0	298.1	300.8	287.4	325.7	230.9
	335.8	308.6	297.7	295.0	294.4	299.9	287.4	295.2	269.3	272.4	310.3	221.5
	351.7	340.8	326.5	330.9	334.9	331.7	326.4	329.0	310.9	297.4	340.8	228.7

Plate 1 - Fluorescent signal (Excitation 544nm/Emission 590nm) 72 hours (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
DU-145	524.3	517.8	345.3	517.8	507.5	412.5	512.0	478.5	363.9	358.0	512.8	39.23
	509.3	500.3	342.1	505.2	502.0	401.1	508.7	466.8	351.0	354.7	493.3	36.86
	509.4	499.8	340.8	505.5	496.6	397.1	491.3	469.6	343.7	350.3	486.9	34.96
	511.8	507.8	349.0	509.5	503.2	400.5	504.9	468.4	348.7	357.8	500.9	37.59
PC-3	488.3	320.9	458.3	307.4	459.8	470.2	461.5	290.1	390.0	440.2	467.6	277.7
	345.1	472.7	281.9	284.9	281.0	287.4	283.1	294.0	226.2	266.0	312.1	149.2
	481.4	332.2	285.5	300.0	297.6	288.1	285.4	299.9	218.6	275.7	318.6	151.0
	358.2	345.0	333.6	324.3	331.5	335.6	320.7	349.4	255.3	384.6	341.8	160.5

Plate 2 - Fluorescent signal (Excitation 544nm/Emission 590nm) 24 hours (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
SK-OV-3	69.15	56.44	49.31	58.43	81.16	58.07	59.04	59.20	61.67	68.78	69.97	64.14
	55.30	60.90	59.43	60.14	59.69	57.29	60.23	52.31	53.64	59.08	51.81	50.25
	54.47	65.33	59.23	60.58	65.62	64.16	64.94	80.70	69.39	69.74	59.49	55.54
	69.74	60.45	54.01	55.25	55.35	57.86	40.91	59.10	42.52	63.85	65.43	55.25
HT-29	33.48	31.63	44.05	39.91	43.91	41.53	42.77	36.16	36.21	38.94	38.56	33.66
	37.67	29.99	36.86	25.68	37.23	30.44	34.83	36.75	30.90	39.74	36.35	36.92
	27.70	32.07	41.67	38.68	40.07	33.52	34.51	35.67	30.76	38.63	34.51	35.62
	36.38	45.13	39.53	33.64	35.92	33.00	33.56	37.26	32.72	41.12	36.29	39.23

Plate 2 - Fluorescent signal (Excitation 544nm/Emission 590nm) 48 hours (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
SK-OV-3	93.82	74.35	70.20	66.11	61.42	78.55	62.22	70.43	61.66	68.33	70.52	44.22
	67.39	55.05	54.93	57.28	52.70	56.15	47.69	62.15	57.88	64.02	68.93	40.62
	67.76	63.60	76.48	65.07	65.51	70.28	81.74	77.29	66.37	89.13	80.84	46.99
	72.59	71.21	57.32	60.48	55.40	69.49	56.40	68.41	61.05	63.84	64.77	40.53
HT-29	45.85	46.76	46.13	44.26	53.77	37.95	42.47	36.90	37.64	48.89	37.71	28.80
	39.13	35.92	42.21	42.35	36.59	42.06	37.39	38.97	29.81	33.52	41.48	32.57
	31.89	32.58	42.34	34.46	36.88	35.60	34.61	38.80	26.14	35.59	40.16	33.57
	41.09	37.10	41.53	39.11	43.36	37.46	40.65	38.31	29.99	41.42	40.08	34.88

Plate 2 - Fluorescent signal (Excitation 544nm/Emission 590nm) 72 hours (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
SK-OV-3	81.81	64.35	76.49	73.01	60.37	58.08	55.95	81.31	64.70	70.47	69.13	33.64
	67.02	67.41	71.16	62.82	69.86	74.66	73.65	80.15	57.87	73.70	87.30	31.52
	72.82	57.45	67.87	61.02	63.17	62.54	52.39	54.31	51.80	81.55	65.70	30.80
	84.13	62.83	70.22	75.83	73.85	74.57	73.03	76.68	50.08	83.84	79.75	33.26
HT-29	49.33	36.42	45.57	37.02	34.49	39.49	36.64	33.00	26.56	39.40	40.03	24.27
	41.73	34.98	42.10	38.66	36.68	36.56	38.54	30.85	25.33	39.75	40.49	24.94
	34.74	40.24	41.83	39.31	38.01	36.17	39.00	38.17	25.64	41.05	45.55	24.24
	46.75	39.15	53.39	48.52	44.59	43.22	42.55	42.08	26.46	47.27	41.92	28.16

Plate 2 - Fluorescent signal (Excitation 544nm/Emission 590nm) 24 hours (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
SK-OV-3	483.5	469.0	462.9	474.5	490.6	476.5	477.7	490.2	481.7	487.9	489.5	439.0
	479.0	478.2	482.7	476.2	483.3	472.4	468.8	467.8	470.2	458.8	461.0	411.5
	472.0	481.0	465.1	485.4	455.9	498.6	477.6	505.7	486.6	479.6	468.9	416.5
	467.4	470.1	457.4	458.5	439.5	447.2	424.4	452.6	403.5	461.0	466.4	403.7
HT-29	245.2	275.7	269.9	253.2	256.2	251.7	243.8	250.7	191.4	261.5	227.6	180.4
	255.9	256.4	269.7	251.9	244.9	256.0	239.3	238.9	190.9	254.3	229.3	184.8
	243.0	257.4	271.7	246.2	254.9	229.1	232.9	242.5	181.8	253.3	219.9	184.7
	216.0	255.5	254.6	238.9	246.4	217.1	210.3	226.8	190.4	269.0	213.1	186.5

Plate 2 - Fluorescent signal (Excitation 544nm/Emission 590nm) 48 hours (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
SK-OV-3	517.1	503.1	489.6	500.3	491.6	502.2	491.4	505.2	490.8	508.5	500.4	394.5
	498.1	499.0	489.7	493.2	494.4	494.4	488.5	493.5	488.5	501.3	487.3	391.6
	497.6	496.0	492.8	492.5	488.8	494.8	486.1	501.4	483.2	504.1	490.3	392.0
	500.1	506.9	484.9	492.9	484.7	493.3	481.4	498.4	482.4	493.9	487.9	387.2
HT-29	183.6	182.1	249.9	201.2	210.2	228.4	224.9	191.6	212.0	293.6	175.6	200.9
	182.2	193.7	248.4	207.1	186.5	224.3	184.6	176.1	202.0	247.4	188.1	201.7
	154.6	180.5	227.7	185.8	183.7	221.7	178.1	189.4	202.4	251.1	172.8	207.1
	160.5	178.8	213.7	174.7	177.5	216.2	190.9	173.6	206.1	251.8	152.0	209.1

Plate 2 - Fluorescent signal (Excitation 544nm/Emission 590nm) 72 hours (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
SK-OV-3	446.4	483.2	482.6	481.3	470.2	481.5	468.7	482.7	470.2	491.0	479.3	259.7
	482.7	490.8	493.3	494.4	482.9	495.0	473.8	488.8	461.7	482.7	484.3	254.3
	500.5	482.2	492.8	489.1	497.4	494.5	471.5	475.3	480.3	499.4	485.4	255.3
	493.7	491.6	495.8	507.7	492.8	499.6	482.6	497.7	460.9	497.1	467.2	256.7
HT-29	64.86	69.54	100.4	66.67	62.05	110.3	79.85	78.43	208.8	175.6	69.42	131.4
	76.17	71.61	102.9	75.30	63.00	129.7	92.43	77.14	202.3	182.5	64.32	136.8
	55.63	56.86	92.87	51.84	60.26	100.1	60.48	74.68	180.5	171.9	51.33	132.9
	76.47	62.04	97.18	60.76	64.60	125.9	84.41	95.98	199.7	215.9	76.70	150.4

Plate 3 - Fluorescent signal (Excitation 544nm/Emission 590nm) T0 (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
Du-145	27.42	27.96	24.09	26.76	24.27	26.09	24.03	26.32	24.80	29.28	26.63	29.30
SK-OV-3	23.11	25.63	26.25	25.39	24.61	24.95	23.57	24.97	24.74	26.65	24.82	27.26
HT-29	20.79	21.14	21.08	20.84	20.84	21.34	21.02	21.61	20.42	20.08	21.07	20.16
PC-3	34.76	28.08	29.25	29.74	30.19	30.03	28.33	28.33	29.51	30.20	31.25	31.73

Plate 3 - Fluorescent signal (Excitation 544nm/Emission 590nm) T0 (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
Du-145	393.5	389.7	375.4	368.5	360.9	366.2	359.8	367.3	350.2	370.3	357.1	361.8
SK-OV-3	324.2	308.1	318.5	316.4	317.0	321.9	313.8	315.6	317.9	314.9	318.1	327.6
HT-29	169.6	156.1	169.6	166.9	174.8	173.8	174.8	168.6	168.0	167.3	163.8	157.7
PC-3	299.8	289.2	293.4	301.3	298.1	305.3	294.8	301.9	293.4	300.5	286.3	290.2

Plate 4 - Fluorescent signal (Excitation 544nm/Emission 590nm) Blank (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
DU-145 Medium	18.58	16.38	17.40	17.04	16.80	16.46	16.59	16.42	17.25	17.75	17.13	17.18
	15.49	16.35	16.82	16.73	16.70	14.75	16.74	16.34	16.24	17.46	16.25	18.22
	16.85	16.94	17.68	17.34	16.82	15.74	17.36	17.52	17.33	17.98	17.54	16.83
	16.63	15.92	17.36	17.11	16.83	16.49	17.44	17.43	15.97	17.73	16.78	17.76
PC-3 Medium	13.40	16.71	16.49	17.77	16.83	16.37	18.52	17.62	15.87	17.35	16.89	18.77
	17.39	16.73	16.14	17.35	16.27	15.63	16.91	15.69	16.13	16.07	16.47	17.62
	17.59	16.80	17.36	14.03	16.86	15.68	18.01	16.16	16.68	17.82	16.62	17.05
	14.57	17.54	16.58	16.36	17.08	16.35	16.94	16.68	16.53	17.27	17.19	16.46

Plate 4 - Fluorescent signal (Excitation 544nm/Emission 590nm) Blank (4hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
SK-OV-3 Medium	19.34	17.81	18.11	18.81	18.19	18.94	19.16	19.47	17.89	18.53	18.28	18.91
	17.44	18.02	17.70	18.47	18.29	18.08	18.80	17.54	18.93	19.29	17.30	17.58
	18.17	18.13	18.46	18.35	18.75	17.06	17.29	18.28	17.80	19.61	17.78	18.46
	17.86	18.04	18.17	16.56	17.65	18.36	18.61	17.32	17.75	17.59	17.07	18.44
HT-29 Medium	16.69	13.24	13.57	15.50	17.52	18.23	18.63	18.71	19.19	16.88	18.27	19.50
	19.07	16.00	15.96	16.17	15.84	16.56	16.02	15.70	16.14	17.55	15.77	16.74
	15.61	16.41	15.94	15.78	16.31	16.32	16.29	16.03	16.33	18.09	16.10	16.58
	16.14	16.23	16.07	15.60	16.08	15.88	16.16	16.02	16.00	17.63	15.38	16.20

Plate 4 - Fluorescent signal (Excitation 544nm/Emission 590nm) Blank (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
<b>DU-145 Medium</b>	22.45	22.02	21.65	21.83	21.41	21.95	21.62	21.84	21.74	22.86	21.82	23.69
	23.07	22.27	21.99	21.84	21.70	21.79	21.87	21.85	21.84	22.57	21.90	23.52
	22.30	21.88	22.09	22.00	21.74	22.07	21.93	21.80	21.58	22.68	21.70	23.54
<b>PC-3 Medium</b>	22.54	21.78	22.02	21.84	21.52	21.96	21.71	22.05	21.64	22.72	21.87	23.46
	21.76	21.98	22.10	21.89	21.65	22.09	22.22	21.77	21.79	22.58	21.68	23.39
	21.90	22.16	22.13	21.99	21.87	21.78	21.92	21.78	21.75	22.63	21.69	23.70
	22.26	22.57	21.99	21.99	21.72	22.01	21.93	22.02	21.81	22.53	21.62	23.69
	22.14	21.94	22.17	21.95	22.08	22.14	22.20	22.18	22.05	22.89	21.89	23.61

Plate 4 - Fluorescent signal (Excitation 544nm/Emission 590nm) Blank (24hr Alamar-Blue™)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
<b>SK-OV-3 Medium</b>	22.20	22.04	21.82	22.03	21.56	21.98	21.86	21.97	21.71	23.03	21.99	22.96
	22.37	22.07	22.01	21.84	21.81	21.85	21.92	21.83	21.81	22.80	21.93	22.92
	22.08	22.03	21.81	21.88	21.70	21.99	21.68	21.89	21.55	22.66	21.56	22.80
<b>HT-29 Medium</b>	21.99	22.12	22.24	21.79	21.64	22.52	21.78	21.86	21.79	22.54	21.63	22.84
	20.68	22.10	23.81	23.62	22.48	22.39	22.36	22.19	22.28	21.59	22.10	22.81
	22.65	20.38	20.17	20.42	20.13	20.52	20.18	20.11	20.54	21.96	20.01	21.65
	20.67	20.66	20.27	20.34	20.00	19.95	20.06	20.09	20.02	22.06	20.03	21.26
	20.68	20.50	20.42	20.45	20.45	20.32	20.54	20.31	20.42	22.47	20.33	21.97