

**SCREENING OF 15 COMPOUNDS VIABILITY AND PROLIFERATION EFFECTS ON U87-MG  
CELL LINES**

*Ng-4 (44), Ng-6 (30), Ng-6A (31), Ng-7 (33), Ng-9 (31), Ng-10 (34), Ng-11 (40), Ng-14 (42), Ng-15 (39),  
Ng-22 (32), OF-3 (55), OF-7 (36),  
Ng-1 (43), Ng-32 (38), Ng-33 (37)*

Study No: IDL/005/CTX

*Technical Report*

Submitted to:  
Idealiza Ltd.  
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**DATA PAGE**

Protocol Signed by the Study Director:	December 19, 2007
Initiation of Study:	December 9, 2007
Completion of Study:	December 13, 2007
HBI Study No.:	IDL/004/CTX
Archive Location:	Harlan Biotech Israel Kiryat Weizmann, Bldg. 13B Rehovot 76326, Israel
Sponsor:	Idealiza Ltd. Israel
Sponsor Monitor:	Dr. Michael Kutushov
Testing Facility:	Harlan Biotech Israel Kiryat Weizmann, Bldg. 13B Rehovot 76326, Israel

Total No. of Pages: 8

## 1. **OBJECTIVE**

The purpose of this study is to screen for viability/proliferation effects of 15 newly developed Test Items on human astrocytoma cell line 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-4	Powder	44	290.32	Idealiza	2-8°C	NA
2	Ng-6	Powder	30	305.83	Idealiza	2-8°C	NA
3	Ng-7	Powder	33	319.83	Idealiza	2-8°C	NA
4	Ng-9	Powder	31	544.2	Idealiza	2-8°C	NA
5	Ng-10	Powder	34	259.7	Idealiza	2-8°C	NA
6	Ng-11	Powder	40	456.6	Idealiza	2-8°C	NA
7	Ng-14	Powder	42	178.23	Idealiza	2-8°C	NA
8	Ng-15	Powder	39	616.5	Idealiza	2-8°C	NA
9	Ng-21	Powder	44	80	Idealiza	2-8°C	NA
10	Ng-22	Powder	32	370.0	Idealiza	2-8°C	NA
11	OF-3	Powder	55	927.0	Idealiza	2-8°C	NA
12	OF-7	Powder	36	479.02	Idealiza	2-8°C	NA
13	Ng-1	Liquid	43	228.2	Idealiza	2-8°C	NA
14	Ng-32	Liquid	38	267.36	Idealiza	2-8°C	NA
15	Ng-33	Liquid	37	67.09	Idealiza	2-8°C	NA

### 2.2 **Vehicle Control Item**

Growth medium + DMSO (0.02%)

### 2.3 **Adjunct Materials:**

2.4.1 Name: AlamarBlue™

Catalog no. BUF012B  
Batch No.: 010507  
Physical state: Liquid  
Supplied by: Serotec  
Storage conditions: 5±3°C  
Expiry Date: August 2008

2.4.2 Name: DMSO  
 Batch No.: 056K0173  
 Catalog No.: D-5879  
 Supplied by: Sigma  
 Physical state: Liquid  
 Storage conditions: Room temperature 18-28°C  
 Expiry Date: August 2008

**2.4 Culture Growth Medium:**

2.5.1 Name: **U87 Growth Medium**  
 Batch: To be specified at the report  
 Content: DMEM supplemented with 2mM glutamine, 10% FBS (Fetal Bovine Serum), Gentamycin  
 Components supplied by: Biological Industries, Israel  
 Storage conditions: 5±3°C  
 Expiry Date: 291107

**2.5 Samples Preparation:**

Test Items are dissolved in 100% DMSO to obtain 10 mM final concentration. Stock solutions are diluted ×5000 with growth medium to achieve working solutions of 2µM. The Vehicle Control solution contains 0.02% DMSO in Growth Medium.

**2.6 Test System**

U87-MG Human brain glioblastoma cell line, ATCC # HTB-14

**2.7 Test procedure:**

U87 cells were cultured under sterile conditions and passed at least twice in its Growth Medium. Then, exponentially growing culture was collected, centrifuged, counted and seeded at the density of 5000 cells/well in pre-warmed Growth Medium in 2×96-well tissue culture plates as per plate plan below. 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.

Thereafter, 100µl of the various treatment solutions were added according to the following plates plan.

Plate 1 – T<sub>0</sub>

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
Alamar Blue was added the day following the seeding of U87 cells, and signal was measured after 1, 2 and 4 hours thereafter.												

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Test Item 1						Test Item 8					
B	Test Item 2						Test Item 9					
C	Test Item 3						Test Item 10					

D	Test Item 4	Test Item 11
E	Test Item 5	Test Item 12
F	Test Item 6	Test Item 13
G	Test Item 7	Test Item 14
H	Vehicle Control	Test Item 15
Plate was incubated with the Test Materials for 72 hours at 37±1°C, 5±0.5% CO <sub>2</sub> and 95±5% humidity, after which Alamar Blue was added. The signal was measured 1, 2 and 4 hours thereafter.		

Following 72 hours of incubation, the treatments were replaced with 100µL/well of AlamarBlue™ (10% in growth medium) and the fluorescent signal (Excitation 544nm/Emission 590nm) was measured following an appropriate incubation time (1, 2 and 4 hours).

### 3. DATA EVALUATION

3.1 Average Optical Density (O.D.) was calculated for each set of replicate wells.

3.2 Cell growth was expressed as (percentage):

a) Percent of t<sub>0</sub>:

$$\frac{\text{Averaged O.D} \times 100}{\text{Averaged O.D at } t_0}$$

b) Percent of Control:

$$\frac{(\text{Averaged O.D} - t_0) \times 100}{(\text{Averaged O.D vehicle} - t_0)}$$

Negative value (<0%) indicates cytotoxic effect

Positive value (0-99%) indicates cytostatic effect.

3.3 Viability (%) was expressed as:

$$\frac{\text{Averaged O.D (treatment)} \times 100}{\text{Averaged O.D (vehicle)}}$$

### 4. RESULTS:

The effect of the Test Items on U87 cell line was evaluated using the AlamarBlue Assay.

**Table 1**

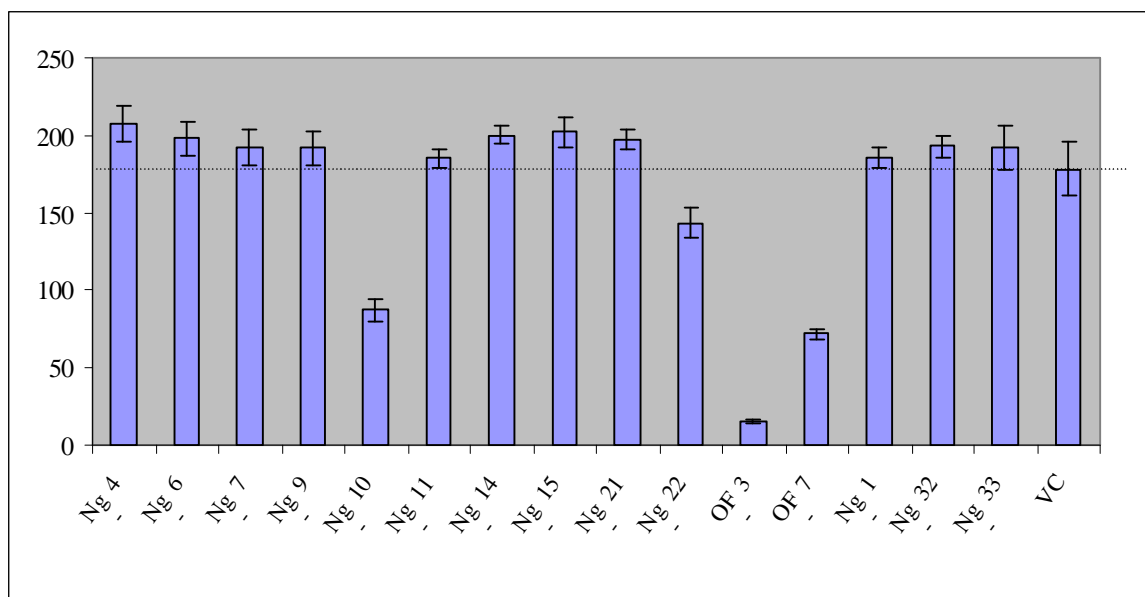
The effect of Test Items on U87 cell line proliferation and viability

Test Item No.	Test Item Name	O.D (544/590nm) Av ±Std'	Percent of t <sub>0</sub> (%)	Percent of Control (%)	Viability (%)	Effect
1	Ng-4	407.1 ± 11.77	207.1	136.8	116.2	None
2	Ng-6	389.4 ± 10.64	198.1	125.3	111.1	None
3	Ng-7	377.2 ± 11.55	191.9	117.4	107.6	None
4	Ng-9	376.5	191.5	116.9	107.4	None

		$\pm 10.96$				
<b>5</b>	Ng-10	<b>171.7</b> $\pm 6.96$	87.4	-16.2	49.0	Cytotoxic
<b>6</b>	Ng-11	<b>363.7</b> $\pm 6.17$	185.0	108.6	103.8	None
<b>7</b>	Ng-14	<b>393.5</b> $\pm 6.16$	200.2	128.0	112.3	None
<b>8</b>	Ng-15	<b>396.5</b> $\pm 9.42$	201.7	129.9	113.1	None
<b>9</b>	Ng-21	<b>387.8</b> $\pm 6.51$	197.3	124.3	110.7	None
<b>10</b>	Ng-22	<b>282.4</b> $\pm 9.36$	143.6	55.7	80.6	Cytostatic
<b>11</b>	OF-3	<b>31.2</b> $\pm 1.22$	15.9	-107.5	8.9	Cytotoxic
<b>12</b>	OF-7	<b>141.1</b> $\pm 3.29$	71.8	-36.1	40.3	Cytotoxic
<b>13</b>	Ng-1	<b>364.9</b> $\pm 5.98$	185.6	109.4	104.1	None
<b>14</b>	Ng-32	<b>379.1</b> $\pm 7.01$	192.8	118.6	108.2	None
<b>15</b>	Ng-33	<b>377.2</b> $\pm 13.88$	191.9	117.4	107.6	None
<b>16</b>	VC	<b>350.4</b> $\pm 71.14$	178.2	100	100	

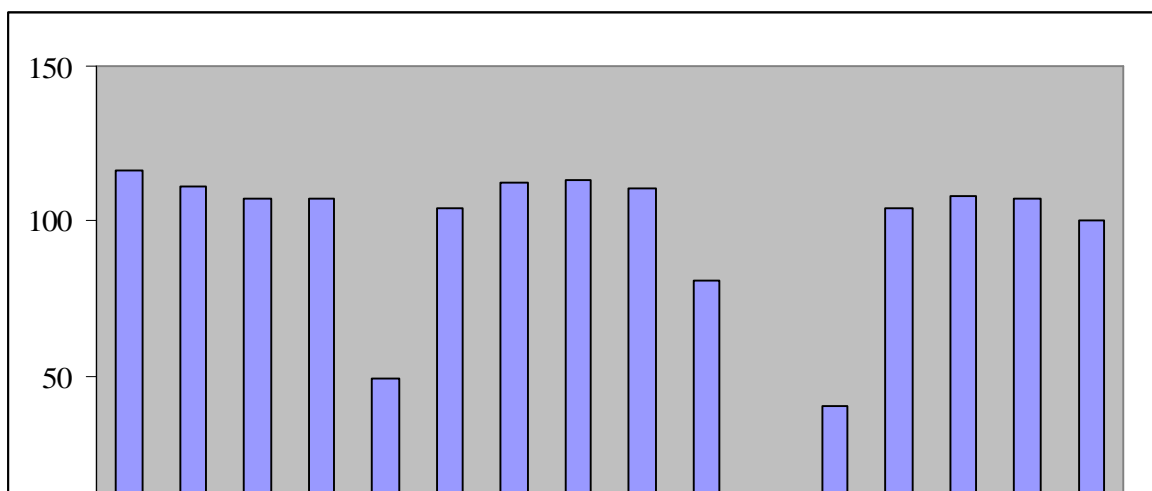
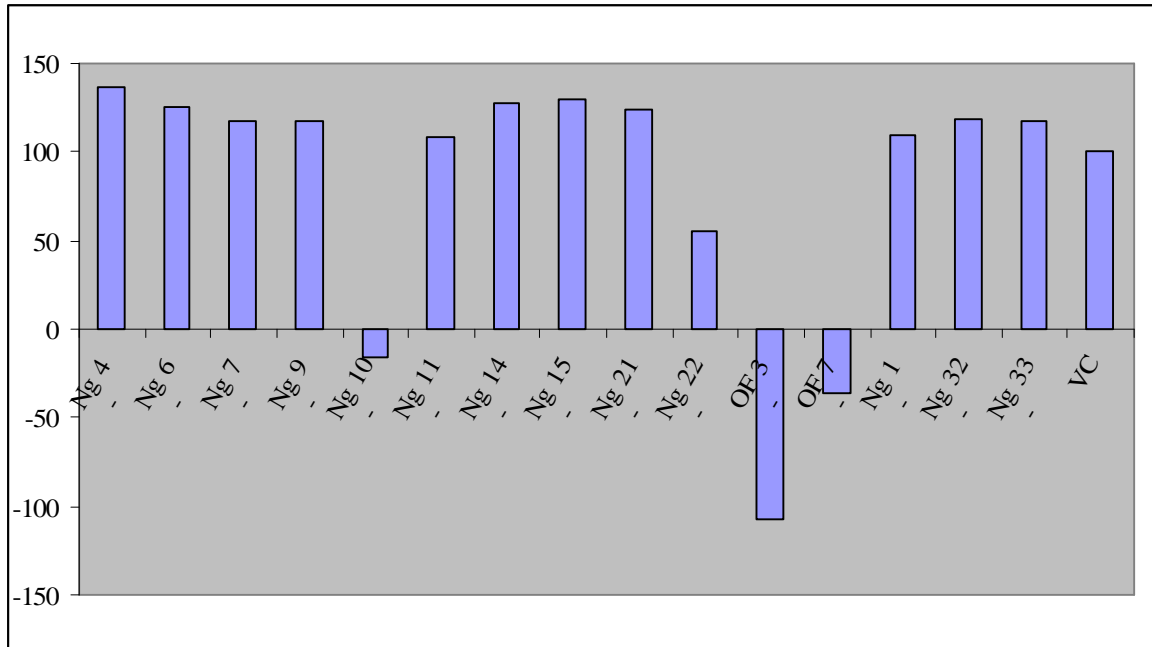
**Figure 1**

The effect of Test Items on the proliferation of U87 cell line expressed as percent of  $t_0$



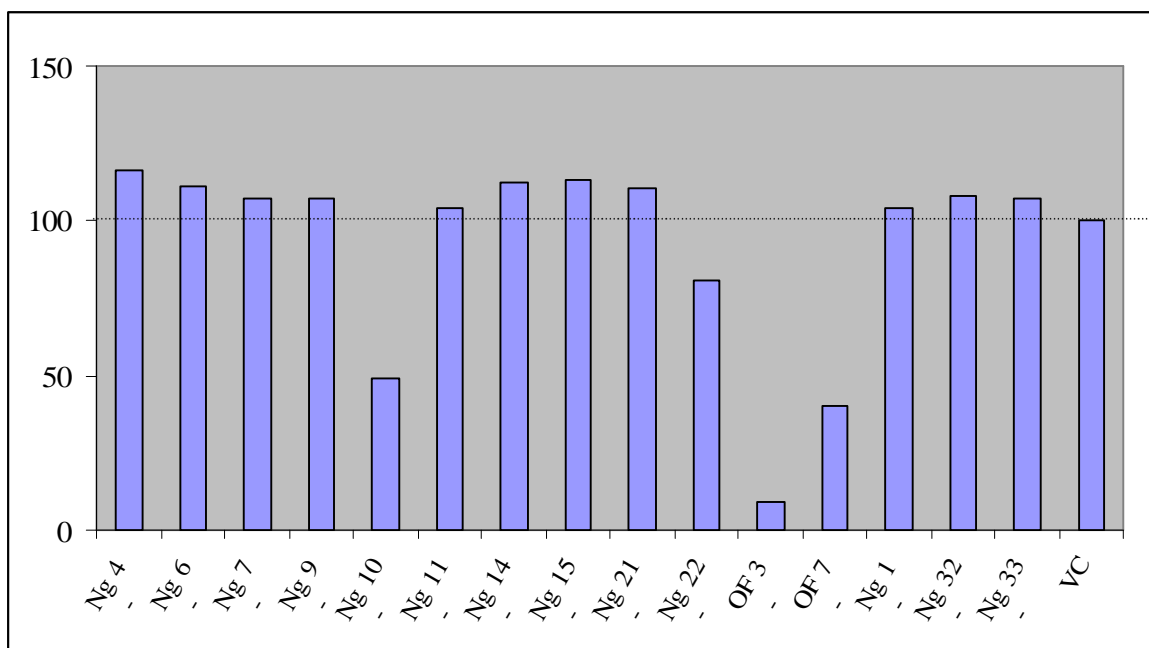
**Figure 2**

The effect of Test Items on the proliferation of U87 cell line expressed as percent of Vehicle Control



**Figure 3**

The effect of Test Items on the viability of U87 cells



**5. CONCLUSIONS:**

The viability of U87 cells was significantly reduced by Ng-10, OF-3, and OF-7 at the concentration of  $10^{-6}$ M (49%, 8.9% and 40.3% respectively) as compared to Vehicle Control, and are thus considered cytotoxic to U87 cells.

The proliferation of U87 cells was slightly (20%) inhibited by Test Item  $10^{-6}$ M Ng-22.

**RAW DATA**

**Plate 1**

1 Hr After the addition of Alamar Blue

	1	2	3	4
A	46.85	38.58	40.58	39.39
B	48.74	42.12	41.08	41.61

2 Hrs After the addition of Alamar Blue

	1	2	3	4
A	76.81	76.33	77.01	78.97
B	83.82	85.78	84.84	91.88

4 Hrs After the addition of Alamar Blue

	1	2	3	4
A	185.8	180.6	183.1	194.0
B	206.4	204.2	201.5	219.3

**Plate 2**

1 Hr After the addition of Alamar Blue

	1	2	3	4	5	6	7	8	9	10	11	12	
TI 1	109.5	78.03	76.34	81.96	79.50	81.11	80.99	80.45	83.14	74.05	80.71	72.03	TI 8
TI 2	83.92	73.07	63.78	62.11	64.80	64.70	70.20	68.30	67.79	71.00	69.96	69.82	TI 9
TI 3	75.16	67.40	70.35	68.02	60.13	57.68	40.28	45.48	39.98	41.43	41.62	46.60	TI 10
TI 4	71.22	57.94	54.28	52.23	51.58	61.59	15.77	16.16	16.24	16.64	15.63	16.72	TI 11
TI 5	34.68	31.10	28.84	27.89	29.44	29.66	27.98	28.21	27.37	28.52	28.12	28.80	TI 12
TI 6	61.21	59.06	49.92	56.33	57.82	54.07	56.90	54.72	56.57	59.96	59.96	65.59	TI 13
TI 7	72.98	70.86	62.01	64.31	65.66	67.17	63.51	63.97	60.96	66.15	63.90	71.91	TI 14
VC	41.03	61.51	66.94	66.00	68.29	63.97	65.75	64.90	58.91	73.09	64.10	65.00	TI 15

2 Hrs After the addition of Alamar Blue

	1	2	3	4	5	6	7	8	9	10	11	12	
TI 1	237.9	215.6	218.9	223.8	222.2	218.7	221.4	217.2	223.2	211.3	213.8	191.8	TI 8
TI 2	200.7	201.9	181.4	182.9	177.1	189.5	192.0	201.4	201.3	207.0	196.3	195.5	TI 9
TI 3	203.0	181.8	176.0	186.3	162.1	170.5	103.4	116.7	105.0	109.5	105.4	120.7	TI 10
TI 4	186.1	168.1	171.9	157.4	156.6	182.2	20.98	20.89	20.89	22.28	20.78	22.26	TI 11
TI 5	72.33	67.66	69.05	62.27	65.09	65.03	59.34	59.62	58.09	61.76	59.28	61.04	TI 12
TI 6	178.7	155.4	163.9	154.4	164.9	158.3	165.8	160.1	165.6	175.1	168.3	184.4	TI 13
TI 7	205.3	192.3	195.2	192.6	192.8	196.3	185.9	186.1	175.0	192.5	181.1	194.1	TI 14
VC	89.84	157.2	199.0	191.6	189.5	187.5	186.6	183.8	160.8	199.9	180.2	176.5	TI 15

4 Hrs After the addition of Alamar Blue

	1	2	3	4	5	6	7	8	9	10	11	12	
TI 1	428.2	398.2	412.2	399.7	406.3	397.8	405.5	398.2	397.9	394.3	403.9	379.2	TI 8
TI 2	406.7	396.4	383.1	382.8	377.8	389.8	387.1	394.9	385.6	396.2	379.6	383.5	TI 9
TI 3	391.1	383.8	373.1	384.0	358.5	372.9	276.5	291.7	276.1	283.9	271.3	294.6	TI 10
TI 4	391.5	378.4	372.5	371.7	360.2	384.7	30.82	30.20	30.14	33.16	30.87	32.29	TI 11
TI 5	180.9	177.4	164.7	172.2	163.0	172.2	141.9	143.8	136.8	145.5	138.2	140.4	TI 12
TI 6	364.6	374.8	358.8	359.8	358.8	365.4	360.4	362.0	363.1	371.4	359.0	373.3	TI 13
TI 7	390.7	402.8	389.6	397.3	385.6	395.1	377.0	387.1	372.1	386.7	370.9	380.5	TI 14
VC	208.7	348.7	381.8	387.9	383.9	391.7	378.0	389.8	356.4	394.0	367.6	377.5	TI 15

