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A Geno Technology, Inc. (USA) brand name

AlamarBlue Cell Viability Assay Reagent

(Cat. # 786-921, 786-922 & 786-923)

INTRODUCTION

The AlamarBlue® Cell Viability Assay Reagent is used to quantify cellular metabolic activity and in turn determine the concentration of viable cells in a given sample. The supplied reagent can be used to measure the cell growth kinetics i.e. the growth of a cell population over time by measuring it at two or more time points of the sample(s).

AlamarBlue Cell Viability Reagent quantitatively measures the proliferation of mammalian cell lines, bacteria and fungi. The dye incorporates an oxidation-reduction (REDOX) indicator that both fluoresces and change color in response to the chemical reduction due to cell growth. Since, the AlamarBlue is very stable and nontoxic to the cells, continuous monitoring of cultures over time is possible.

The alamarBlue dye in its oxidized form is blue in color and non-fluorescent. In alamarBlue Reagent assay, the growing cells cause a chemical reduction of the alamarBlue dye from non-fluorescent blue to fluorescent red. The continued growth of viable cells maintain a reducing environment (fluorescent, red) and inhibition of growth maintains an oxidized environment (non-fluorescent, blue), which can be detected using a fluorescence or absorbance detector.

Conversion of alamarBlue dye from oxidized to reduced form

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-921	AlamarBlue Cell Viability Reagent	10ml
786-922	AlamarBlue Cell Viability Reagent	25ml
786-923	AlamarBlue Cell Viability Reagent	50ml

STORAGE CONDITION

It is shipped at ambient temp. Upon arrival, store it at 4°C, protected from light. The reagent is stable for six months, when stored and used as recommended.

ADDITIONAL ITEM(S) REQUIRED

- Fluorescence plate reader with excitation 530-570nm and emission 580-620nm
- Microtiter plate reader for reading absorbance at 570nm and 600nm
- Multi-channel Pipettor
- 96 well tissue culture plates, compatible with fluorometer
- Culture plate shaker

PREPARATION BEFORE USE

- Allow the reagent to come to room temperature.
- To prepare 100% reduced form of alamarBlue Reagent to use as Positive Control in fluorescence measurements, autoclave a sample containing cell culture media and alamarBlue for 15 minutes

IMPORTANT INFORMATION

1. The alamarBlue dye present in the supplied reagent and its reduced form converted during the assay are light sensitive. Prolonged exposure of the reagent to light will result in increased background fluorescence in the assay and decreased sensitivity.
2. Media containing reagents with high redox potential such as DTT must not be used.

ASSAY CONTROLS

The following assay controls are recommended for each assay plate set up:

Negative Control	**Untreated Control**	**Positive Control (100% Reduced)**
No Cell Control may be set up without the cells. This will serve as the Negative Control to determine background fluorescence that might be present.	Untreated Control may be set up with untreated cells. Same solvent used to deliver the test compound may be added to the control wells.	Positive Control with 100% reduced alamarBlue is necessary to assay viability using fluorescence values. To prepare 100% reduced form of alamarBlue Reagent, autoclave a sample containing cell culture media and alamarBlue for 15 minutes

CELL VIABILITY ASSAY PROTOCOL

Protocol for assaying cell viability with a test compound:

1. When cells are in log phase of growth, harvest them and determine the cell count. The suggested optimal cell count is 1 x 10^4 cells/ml (cell density), which may vary between the cell lines.

2. To appropriate wells of a 96 well plate, add the test compound and vehicle controls so that the final volume is 100µl in each well. Culture cells at 37°C in a cell culture incubator for desired test compound exposure period.

3. Remove the assay plates from 37°C incubator and mix by gently shaking it.

4. Aseptically add 10µl of alamarBlue Reagent in an amount equal to 10% of the volume in the well. In the Positive Control well, add 10µl of ultrapure sterile water.

5. Incubate cultures with alamarBlue Reagent for the predetermined time in a cell culture incubator at 37°C (the optimum incubation time may vary between the cell types).

6. After the incubation time ends, remove the plate and measure the fluorescence with Excitation wavelength at 530-560nm and Emission wavelength at 590nm.

7. Alternatively, absorbance at 570nm and 600nm wavelengths can be measured

CALCULATION OF RESULTS

1. To calculate the % Reduction of alamarBlue Reagent with fluorescence based readings, subtract the average fluorescence reading (RFU) of the Untreated (UT) Control from fluorescence values (RFU) of experimental wells as below:

$$\% \text{ Reduction of alamarBlue} = \frac{(\text{Experimental RFU value- Untreated Control RFU value}) \times 100}{100\% \text{ Reduced (+) control RFU} - \text{Untreated control RFU value}}$$

2. To calculate % Difference between Treated (T) & Untreated (UT) Control cells based on fluorescence (RFU values), divide Experimental RFU value with Untreated Cell Control RFU value as below:

$$\% \text{ Difference between T \& UT} = \frac{\text{Experimental RFU value with test compound} \times 100}{\text{Untreated Control RFU value}}$$

3. To calculate the % Reduction of alamarBlue Reagent with absorbance based readings, follow the equation below and use Molar Extinction Coefficient from the table.

$$\% \text{ Reduction of alamarBlue} = \frac{(O2 \times A1) - (O1 \times A2) \times 100}{(R1 \times N2) - (R2 \times N1)}$$

Where:

O1 = Molar Extinction Coefficient of OXIDIZED alamarBlue at 575nm
O2 = Molar Extinction Coefficient of OXIDIZED alamarBlue at 600nm
R1 = Molar Extinction Coefficient of REDUCED alamarBlue at 570nm
R2 = Molar Extinction Coefficient of REDUCED alamarBlue at 600nm
A1 = Absorbance value of test wells at 570nm
A2 = Absorbance value of test wells at 600nm
N1 = Absorbance value of Negative Control well at 570nm
N2 = Absorbance value of Negative Control well at 600nm

Molar Extinction Coefficient of alamarBlue at different wavelengths:

Wavelength	Reduced (R)	Oxidized (O)
540nm	104395	47619
570nm	155677	80586
600nm	14652	117216
630nm	5494	34798

4. To calculate % Difference between Treated (T) & Untreated (UT) Control cells based on absorbance readings, follow the equation below and use Molar Extinction Coefficient from the table on previous page (6).

$$\% \text{ Difference between T \& UT} = \frac{(O2 \times A1) - (O1 \times A2) \times 100}{(O2 \times P1) - (O1 \times P2)}$$

Where:

O1 = Molar Extinction Coefficient of OXIDIZED alamarBlue at 575nm

O2 = Molar Extinction Coefficient of OXIDIZED alamarBlue at 600nm

A1 = Absorbance value of test wells at 570nm

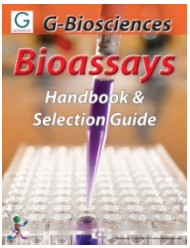
A2 = Absorbance value of test wells at 600nm

P1 = Absorbance value of Untreated Control well at 570nm
(Cells + alamarBlue Reagent and NO Test Agent)

P2 = Absorbance value of Untreated Control well at 600nm
(Cells + alamarBlue Reagent and NO Test Agent)

RELATED PRODUCTS

Download our Bioassays Handbook.



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