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Alamar Blue Cell Viability Reagent

Cat# CC1021 – 5 ml (500 rxn) / CC1022 – 10 ml (1000 rxn) / CC1023 – 30 ml (3000 rxn)

alamarBlue is stable at 4°C with protection from light.

INTRODUCTION

The **Alamar Blue 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 Alamar Blue Cell Viability Reagent has broad applicability and can be used with various human and animal cell lines, bacteria, plant, and fungi. Since, the alamarBlue dye is very stable and nontoxic to the cells, 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).

The continued growth of viable cells maintain a reducing environment and inhibition of growth maintains an oxidized environment. When added to cells, Alamar Blue Cell Viability Reagent is modified by the reducing environment of viable cells and turns red in color and becomes highly fluorescent. This color change and increased fluorescence can be detected using absorbance (detected at 570 and 600 nm) or fluorescence (using an excitation between 530 and 560 and an emission at 590 nm).

CONTENTS

Component	CC1021	CC1022	CC1023
Alamar Blue Cell Viability Assay Reagent	5 ml (500 rxn)	10 ml (1000 rxn)	30 ml (3000 rxn)

PROTOCOL

1. Dispense 100 µl of cell suspension (100-10,000 adherent cells/well or 2,000-50,000 suspension cells/well) in a 96-well plate filled the edged wells with Sterile water or PBS. Set blank wells (There is no cell but with normal culture medium), control wells (without drug treatment) and positive control wells (100% reduced alamarBlue, see note No. 3). Set 3-5 repeat wells in each group. The optimum cell density may vary between cell types.
2. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37°C, 5% CO_2).
3. Add various concentrations of tested substances to the plate and incubate at 37°C for desired exposure period.
4. Mix by gently shaking and then aseptically add alamarBlue reagent in an amount equal to 10% of the volume in the well, i.e. add 10 µl of alamarBlue reagent in the well. In the Positive Control wells, add 10 µl of ultrapure sterile water.
5. Incubate cultures at 37°C for 2 - 6 hours. The optimum incubation time may vary between cell types.
6. Measure the fluorescence with Excitation wavelength at 530-560 nm and Emission wavelength at 590 nm. Alternatively, the absorbance at 570 and 600 nm can also 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:

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

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 575 nm

O2 = Molar Extinction Coefficient of OXIDIZED alamarBlue at 600 nm

R1 = Molar Extinction Coefficient of REDUCED alamarBlue at 570 nm

R2 = Molar Extinction Coefficient of REDUCED alamarBlue at 600 nm

A1 = Absorbance value of test wells at 570 nm

A2 = Absorbance value of test wells at 600 nm

N1 = Absorbance value of Negative Control well at 570 nm

N2 = Absorbance value of Negative Control well at 600 nm

Molar Extinction Coefficient of alamarBlue at different wavelengths:

Wavelength	Reduced (R)	Oxidized (O)
540 nm	104395	47619
570 nm	155677	80586
600 nm	14652	117216
630 nm	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 previous table.

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

Where:

O1 = Molar Extinction Coefficient of OXIDIZED alamarBlue at 575 nm

O2 = Molar Extinction Coefficient of OXIDIZED alamarBlue at 600 nm

A1 = Absorbance value of test wells at 570 nm

A2 = Absorbance value of test wells at 600 nm

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

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

Note :

1. If store at -20°C, need to **avoid repeated thawing and freezing** since repeated thawing and freezing causes an increase in the background, which interferes with the assay. Recommended small dose packing and keep the kit from light with dark or black foil bag.
2. 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.
3. To prepare 100% reduced form of alamarBlue Reagent to use as **Positive Control** in fluorescence measurements, autoclave a sample containing culture media and alamarBlue for 15 minutes.
4. Media containing reagents with high redox potential such as DTT **must not be used**.
5. Prepare the tested drug with culture medium or PBS.
6. Try to avoid destroying cells; manipulation to the cells should be careful.
7. The incubated time with alamarBlue is dependent on the type and number of cells.
8. If 96-well plate is not used for this assay, please calculate the number of cells per well accordingly.

PRODUCT USE LIMITATION

These products are intended for research use only.