AlamarBlue Cell Viability Assay Reagent

measure the viability of mammalian cell lines, bacteria and fungi

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

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AlamarBlue Cell Viability Assay Reagent

Catalog No.: AR4002, AR4002-1

Introduction

The Boster AlamarBlue Cell Viability Assay Reagent is used to quantitatively measure the viability of mammalian cell lines, bacteria and fungi by incorporating a rapid, sensitive and reliable fluorometric/colorimetric growth indicator. AlamarBlue detects cell viability by converting from a non-fluorescent dye to the highly red fluorescent dye resorufin in response to chemical reduction of growth medium resulting from cell growth. The assay is homogenous, requires no cell lysis or washing and offers a simple, rapid, reliable, sensitive, safe and cost-effective measurement of cell viability. The AlamarBlue Reagent allows for viability studies of chemical inhibitors on tumor cell lines and toxicology research to establish baseline data for predicting the toxicity of related novel agents by comparing such baseline data with known in vivo toxicity. Viable cells maintain a reducing environment within their cytoplasm. The AlamarBlue Reagent is an oxidized form of redox indicator that is blue in color and non-fluorescent. When incubated with viable cells, the reagent changes color from blue to red and becomes fluorescent (Figure 1). This change can be detected using fluorescence or absorbance measurement (Figure 2).

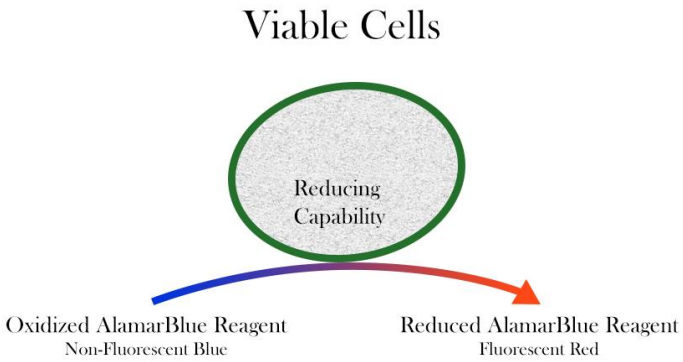

Figure 1: Schematic Representative of AlamarBlue Cell Viability Reagent Undergoing Reduction within the Cells

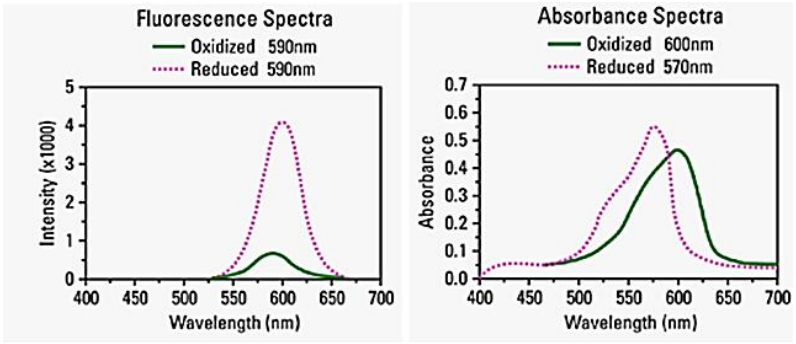

Figure 2: Fluorescence and Absorbance spectra of AlamarBlue Reagent in oxidized and reduced states

Item(s) Supplied:

Catalog No.	Product Name	Size	Storage Condition*
AR4002	AlamarBlue Cell Viability Assay Reagent	10 ml	4°C
AR4002-1	AlamarBlue Cell Viability Assay Reagent	20 ml	4°C

**The product is shipped at ambient temperature and upon receipt, store it at 4°C for long-term storage. The product is stable for one year, if stored and used as recommended.*

Additional Materials Needed but Not Supplied

- Sterile, ultrapure water
- Cultured cell line
- Tissue culture-compatible 96- or 384-well plates compatible with fluorometer and absorbance reading (clear or solid bottom)
- Multichannel pipette
- Fluorescent reader with excitation of 530-560nm and emission of 590nm
- Spectrophotometer plate reader capable of reading 570/600nm absorbance

  Note: Alternative wavelengths of 570/630nm or 540/600nm can be used; do not use a 540/630nm reading.
- Positive control: 100% reduced form of AlamarBlue Reagent. To prepare the reduced reagent form, autoclave AlamarBlue Reagent in culture media (1 volume of AlamarBlue Reagent: 10 volumes of mammalian cell culture media containing serum) for 15 minutes of liquid autoclave cycle.

  Note: *Do not autoclave concentrated AlamarBlue Reagent or AlamarBlue Reagent in phosphate-buffered saline (PBS); 100% reduced AlamarBlue Reagent is not stable in PBS.*

Procedure Summary

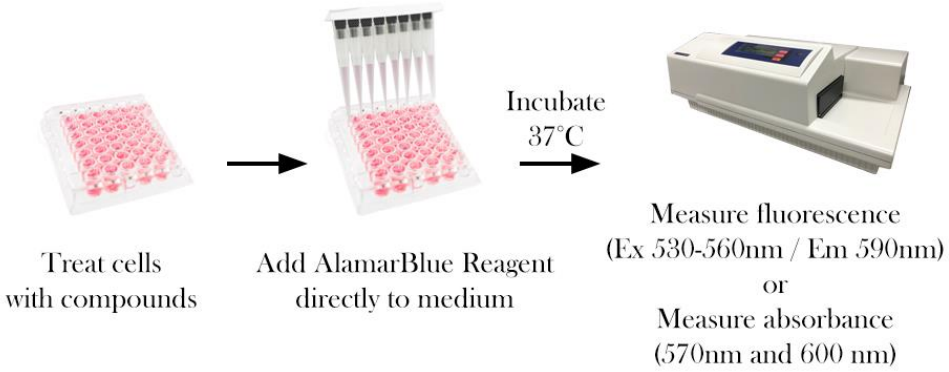

Figure 3: Procedure summary schematic

Important Product Information

- Store the assay protected from light.
- Do not use the assay with media containing reagents with high redox potential (e.g., DTT).
- Procedures are written for 96-well plates; for 384-well plates, reduce all volumes by four-fold.

Determination of Optimum Incubation Time and Cell Number

Note: Cellular response within the assay is highly dependent on the incubation time and the number of cells plated. For best results, determine the optimal incubation time and plating density for each different cell line.

1. Prepare a log phase growth of cells in 100µL of media with cell numbers above and below the cell density expected to be used. Note: Include a negative control of only medium without cells to determine background signal. Include a positive control of 100µL of 100% reduced AlamarBlue Reagent without cells.
2. Add 10µL of AlamarBlue Reagent in an amount equal to 10% of the volume in the well. For the positive control well, add 10µL of sterile, ultrapure water.
3. Incubate the plate in an incubator at 37°C, 5% CO2.
4. Remove the plate and measure fluorescence with excitation wavelength at 530-560nm and emission wavelength at 590nm (refer to Step 5 for calculation) or absorbance at a wavelength of 570nm and 600nm (refer to Step 6 for calculation). Note: Two alternative absorbance wavelength pairs of 570/630nm or 540/600nm can be used (refer to Step 6 for calculation).
5. To calculate the % Reduction of AlamarBlue Reagent using fluorescence readings:

$$\%\ \text{Reduction of alamarBlue Reagent} = \frac{(\text{Experimental RFU value} - \text{Negative control RFU value})}{(\text{100\% reduced positive control RFU value} - \text{Negative control RFU value})} \times 100$$

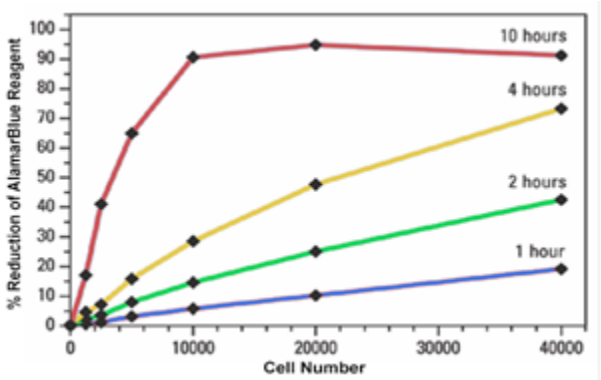

Figure 4. Graph showing the percentage reduction of Alamar Blue Reagent at different cell numbers and incubation hours. Serial dilutions of CHO-K1 cells were plated at 100µL/well in a 96-well plate and incubated at 37°C with 5% CO_2. The Alamar Blue Reagent (10µL/well) was added and the cells were measured at 545nm Excitation/590nm Emission, using Fluorescence Plate Reader.

6. From Figure 4, if the desired incubation time with AlamarBlue Reagent was 4 hours, any plating density from 5000 to 40,000 cells/mL could be used and expected to produce a reaction within the linear range for that incubation period. However, if the intent is to incubate for 10 hours, the reaction could be expected to be within the linear range if plated at 1250 to 10,000 cells/mL for this cell line. There is no longer a linear relationship with the percentage of reduction above 10,000 cells/mL.

   Note: *Microbial contaminants will also reduce AlamarBlue Reagent and yield erroneous results if contaminated cultures are tested by this method.*

   To calculate the % Reduction of AlamarBlue Reagent using absorbance readings:

$$\%\ \text{Reduction of alamarBlue Reagent} = \frac{(E_{oxi}600 \times A_{570}) - (E_{oxi}570 \times A_{600})}{(E_{red}570 \times C_{600}) - (E_{red}600 \times C_{570})} \times 100$$

   Eoxi570 = molar extinction coefficient (E) of oxidized AlamarBlue Reagent at 570nm = 80586
   Eoxi600 = E of oxidized AlamarBlue Reagent at 600nm = 117216
   A570 = absorbance of test wells at 570nm
   A600 = absorbance of test wells at 600nm
   Ered570 = E of reduced AlamarBlue at 570nm = 155677
   Ered600 = E of reduced AlamarBlue at 600nm = 14652
   C570 = absorbance of negative control well (media, AlamarBlue Reagent, no cells) at 570nm
   C600 = absorbance of negative control well (media, AlamarBlue Reagent, no cells) at 600nm

   Note: *Use different values for E for alternative wavelengths of 540/600nm and 570/630nm.*
   Eoxi540 = E of oxidized AlamarBlue Reagent at 540nm = 47619
   Eoxi630 = E of oxidized AlamarBlue Reagent at 630nm = 34798
   Ered540 = E of reduced AlamarBlue Reagent at 540nm = 104395
   Ered630 = E of reduced AlamarBlue Reagent at 630nm = 5494

Cell Viability Assay with Chemical Compounds

1. Harvest log phase-growth cells. Plate cells according to previously determined optimum cell density.
   Note: Include (1) a medium control without cells to determine absorbance of negative control; (2) an untreated cell control to serve as a vehicle control by adding the same solvent used to dissolve the chemical compound or growth factor; (3) a 100% reduced AlamarBlue positive control.
2. Add a chemical compound and vehicle control to the well.
3. Incubate cells for the required time in an incubator at 37°C, 5% CO2.
4. Add 10µL of AlamarBlue Reagent in an amount equal to 10% of the volume in the well. For the positive control well, add 10µL of sterile, ultrapure water.
5. Incubate cells for the pre-determined time in an incubator at 37°C, 5% CO2.
6. Remove the plate and measure fluorescence with excitation wavelength at 530-560nm and emission wavelength at 590nm (refer to Step 7 or 8 for calculation). Alternatively, measure absorbance at a wavelength of 570nm and 600nm (refer to Step 8 for calculation).
   Note: Alternative wavelengths of 570/630nm or 540/600nm can be used (refer to Step 7 for calculation).
7. To calculate the % Reduction of AlamarBlue Reagent using fluorescence-based readings:
8. $$\%\ \text{Reduction of alamarBlue Reagent} = \frac{(\text{Experimental RFU value} - \text{Negative control RFU value})}{(\text{100\% reduced positive control RFU value} - \text{Negative control RFU value})} \times 100$$

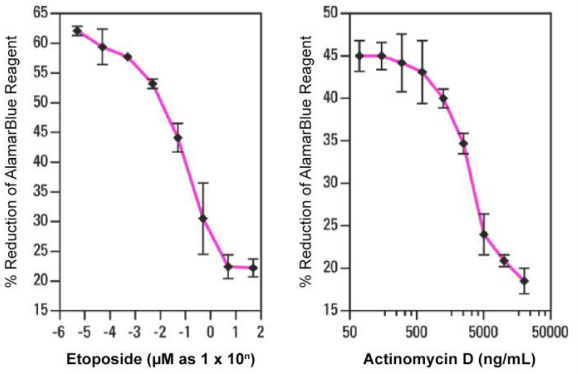

Figure 5. Determination of cell viability using AlamarBlue Cell Viability Reagent. HEK-293 or CHO-K1 cells were plated in a 96-well plate and exposed to different concentrations of Etoposide and Actinomycin D, respectively. Alamar Blue Reagent was added and the cells were incubated at 37°C with 5% CO_2, and the plates were measured at 545nm Excitation /590nm Emission using the Fluorescence Plate Reader.

9. To calculate the % Difference between treated and untreated control cells based on fluorescence readings:

$$\% \text{ Difference between treated and untreated cells} = \frac{\text{Experimental RFU value with chemical compound}}{\text{Untreated cell control RFU value}} \times 100$$

10. To calculate the percentage reduction of AlamarBlue Reagent using absorbance readings:

$$\% \text{ Reduction of alamarBlue Reagent} = \frac{(E_{oxi}600 \times A_{570}) - (E_{oxi}570 \times A_{600})}{(E_{red}570 \times C_{600}) - (E_{red}600 \times C_{570})} \times 100$$

Eoxi570 = E of oxidized AlamarBlue Reagent at 570nm = 80586
Eoxi600 = E of oxidized AlamarBlue Reagent at 600nm = 117216
A570 = absorbance of test wells at 570nm
A600 = absorbance of test wells at 600nm
Ered570 = E of reduced AlamarBlue Reagent at 570nm = 155677
Ered600 = E of reduced AlamarBlue Reagent at 600nm = 14652
C570 = absorbance of negative control well (media, AlamarBlue Reagent, no cells) at 570nm
C600 = absorbance of negative control well (media, AlamarBlue Reagent, no cells) at 600nm

Note: Use different values for E for alternative wavelengths of 540/600nm and 570/630nm.
Eoxi540 = E of oxidized AlamarBlue Reagent at 540nm = 47619
Eoxi630 = E of oxidized AlamarBlue Reagent at 630nm = 34798
Ered540 = E of reduced AlamarBlue Reagent at 540nm = 104395
Ered630 = E of reduced AlamarBlue Reagent at 630nm = 5494

Troubleshooting

Problem	Possible Cause	Solution
Low fluorescence value	Low instrument gain setting	Adjust the instrument's gain settings
	Improper instrument filter / wavelength	Verify the instrument's filter/wavelength setting
	Short incubation time	Increase incubation time
	Low number of cells	Increase cell number
High fluorescence value	High instrument gain setting	Adjust the instrument's gain setting
	Improper instrument filter/wavelength	Verify the instrument's filter / wavelength setting
	Long incubation time	Decrease incubation time
	High number of cells	Decrease cell number
	Bacterial contamination	Identify and remove the contamination source
	AlamarBlue Reagent was exposed to direct light for extended time	Purchase new AlamarBlue reagent after comparing current with previous readings of negative control