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Cell-Quant™ AlamarBlue Cell Viability Reagent

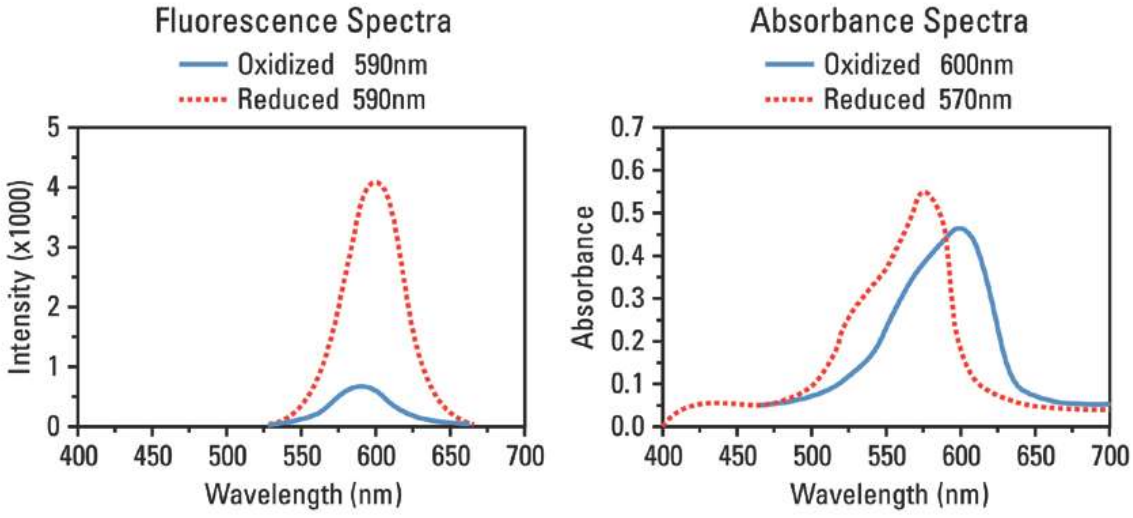
Table 1. Product Package and Storage

Cat No.	Product Name	Amount	Storage
A016-1	AlamarBlue reagent	5 mL	4°C: one year -20°C: two year Protect from light
A016-2	AlamarBlue reagent	25 mL	

Introduction

AlamarBlue Cell Viability Assay Kit offers a simple, rapid, reliable, sensitive, safe and cost-effective measurement of cell viability. AlamarBlue detects cell viability by converting from a nonfluorescent dye to the highly red fluorescent dye resorufin in response to chemical reduction of growth medium resulting from cell growth. Continued cell growth maintains a reduced environment while inhibition of growth maintains an oxidized environment. Reduction related to growth causes the REDOX indicator to change from the oxidized (nonfluorescent, purple color) form to the reduced (fluorescent, red color) form. The fluorescent signal is monitored using 530-560 nm excitation wavelength and 590 nm emission wavelength. The absorbance is monitored at 570 nm and 600 nm. For optimal result, subtract background OD at 600 nm from OD at 570 nm. The fluorescent and colorimetric signal generated from the assay is proportional to the number of living cells in the sample.

AlamarBlue Cell Viability Assay is as sensitive as [3H] thymidine assay for detecting cell proliferation. Depending on the cell types, AlamarBlue can detect as few as 100 cells with reproducible and sensitive signal. As resorufin (pink and fluorescent) can be further reduced to hydroresorufin (colorless and nonfluorescent), the assay signal decreases even with increased number of cells after all resazurin is converted into resorufin. Therefore, it is important to conduct a cell number titration assay for each particular cell line of your interest to identify the optimal number of cells for your assay to avoid this potential problem.



Fluorescence (left) and absorbance (right) spectra of AlamarBlue Reagent in oxidized and reduced states.

Materials required but not provided

• Cultured cell line

• Tissue culture-compatible 96- or 384-well plates compatible with fluorometer and absorbance reading

• Multichannel pipette

• Fluorescent plate reader with excitation of 530-560nm and emission of 590nm

• Spectrophotometer plate reader capable of reading 570/600nm absorbance

• 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.

Assay Protocol

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.

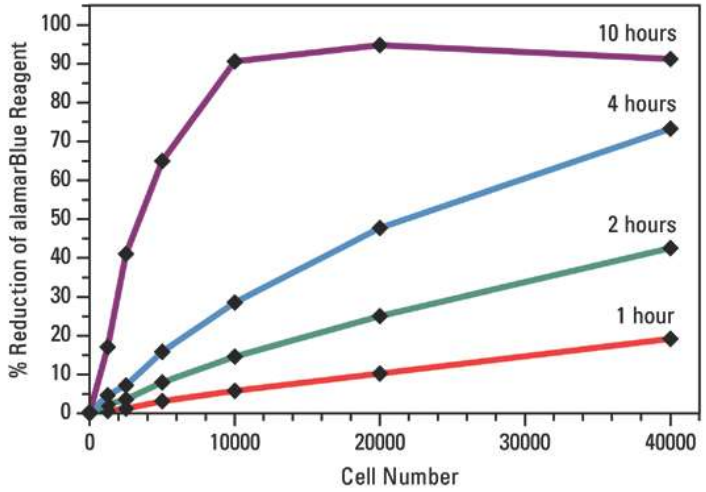
3. Incubate the plate in an incubator at 37°C, 5% CO_2.

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:

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



Graph showing the percentage reduction of AlamarBlue 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, 5% CO_2. The AlamarBlue Reagent (10µL/well) was added and the cells were measured at Ex/Em=545nm/590nm using a Fluorescent Plate Reader.

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

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

$E_{oxi}570$ = molar extinction coefficient (E) of oxidized AlamarBlue at 570nm = 80586
$E_{oxi}600$ = molar extinction coefficient (E) of oxidized AlamarBlue at 600nm = 117216
$E_{red}570$ = molar extinction coefficient (E) of reduced AlamarBlue at 570nm = 155677
$E_{red}600$ = molar extinction coefficient (E) of reduced AlamarBlue at 600nm = 14652
A_{570} = absorbance of test wells at 570nm
A_{600} = absorbance of test wells at 600nm
C_{570} = absorbance of negative control well (media, AlamarBlue Reagent, no cells) at 570nm
C_{600} = absorbance of negative control well (media, AlamarBlue Reagent, no cells) at 600nm
Note: Use different values for molar extinction coefficient (E) for alternative wavelengths of 540/600nm and 570/630nm.
$E_{oxi}540$ = molar extinction coefficient (E) of oxidized AlamarBlue at 540nm = 47619
$E_{oxi}630$ = molar extinction coefficient (E) of oxidized AlamarBlue at 630nm = 34798
$E_{red}540$ = molar extinction coefficient (E) of reduced AlamarBlue at 540nm = 104395
$E_{red}630$ = molar extinction coefficient (E) of reduced AlamarBlue at 630nm = 5494.

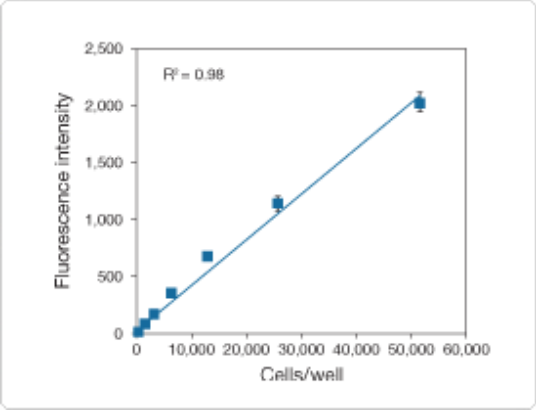
Cell Titration Standard Curve

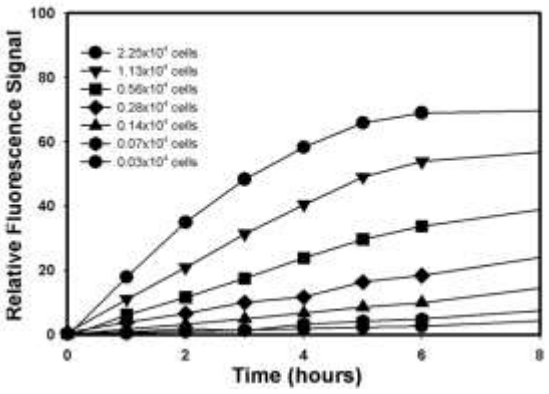
1. Plate cells in 100μL medium into 96-well tissue culture plates by conducting cell number titration in the range of 100 to 10,000 for adherent cells and 2,000 to 500,000 for suspension cells. For background control, use 100μL medium without cells.

2. Add 10μL AlamarBlue reagent into medium and incubate cells for at least 1 hour and up to 24 hours at 37°C.

3. Measure absorbance at 570 nm and 600 nm or fluorescence with excitation wavelength at 530 nm and emission wavelength at 590 nm using a micro-titer plate reader.

4. Obtain OD_{570}-OD_{600} for each sample if colorimetric detection method is chosen, or fluorescence signal from each sample deducted by background fluorescence from the background control, and plot a standard curve to identify the optimal cell concentration for your assay.





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% CO_2.

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.

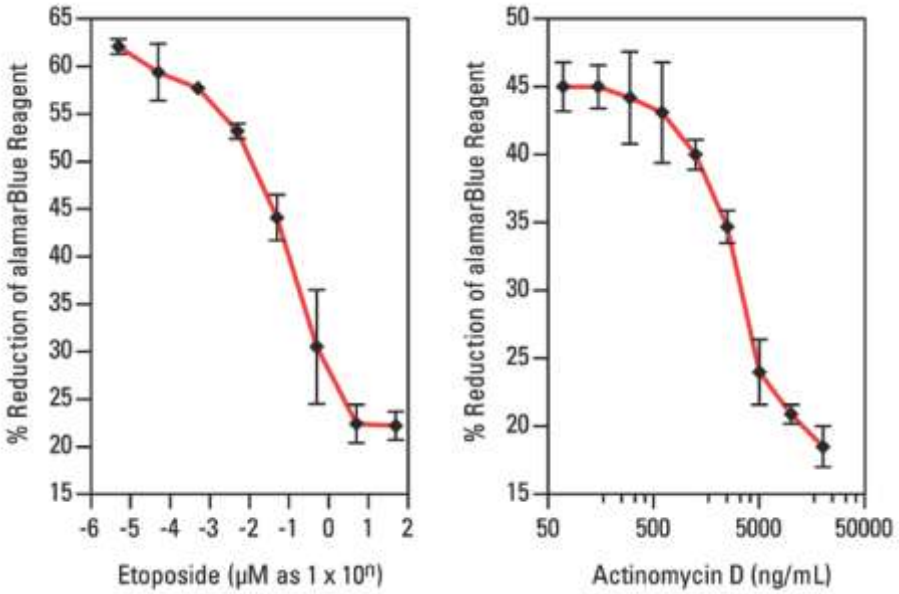
5. Incubate cells for the pre-determined time in an incubator at 37°C, 5% CO_2.

6. Remove the plate and measure fluorescence with excitation wavelength at 530-560nm and emission wavelength at 590nm (refer to Step 7 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 8 for calculation).

7. To calculate the % Reduction of AlamarBlue Reagent using fluorescence-based readings:

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



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 various concentrations of Etoposide and Actinomycin D, respectively. Cells were loaded with alamarBlue Reagent, incubated at 37°C, 5% CO_2, and the plates were measured at Ex/Em=545nm/590nm using a Fluorescent Plate Reader.

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

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

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Troubleshooting

Problem	Possible Cause	Solution
Low fluorescence value	Low instrument gain setting	Adjust the instrument's gain setting
	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