IMMUNOLOGICAL SCIENCES Product Data Sheet

ALAMAR BLUE CELL VIABILITY ASSAY KIT

IK11139-25	Alamar Blue Assay Kit	25 ml	2,500 assays
IK11139-100	Alamar Blue Assay Kit	100ml	10,000 assays

Spectral Properties
After reduction of resazurin to resorufin (at neutral pH):
Absorbance/excitation: 571 nm
Emission: 585 nm

Introduction

Cell health can be monitored by numerous methods. Plasma membrane integrity, DNA synthesis, DNA content, enzyme activity, presence of ATP, and cellular reducing conditions are known indicators of cell viability and cell death.

Alamar Blue cell viability reagent functions as a cell health indicator by using the reducing power of living cells to quantitatively measure the proliferation of various human and animal cell lines, bacteria, plant, and fungi allowing you to establish relative cytotoxicity of agents within various chemical classes. When cells are alive they maintain a reducing environment within the cytosol of the cell.

Resazurin, the active ingredient of Alamar Blue reagent, is a non-toxic, cell permeable compound that is blue in color and virtually non-fluorescent.

Upon entering cells, resazurin is reduced to resorufin, a compound that is red in color and highly fluorescent. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and color of the media surrounding cells.

AlamarBlue cell viability reagent is used to assess cell viability by simply adding the 10X, ready-to-use solution to mammalian or bacterial cells in culture media.

There is no requirement to aspirate media from cells or place cells in minimal media. Consequently, Alamar Blue reagent can easily be used in a single tube or microtiter plate format in a “no wash” fashion.

Brief Procedure:

Add 10 µL Alamar Blue reagent to 100 µL sample, simply add Alamar Blue reagent as 10% of the sample volume followed by a 1–4 hours incubation at 37ºC.

Longer incubation times may be used for greater sensitivity without compromising cell health. The resulting fluorescence is read on a plate reader or fluorescence spectrophotometer.

Alternatively, the absorbance of Alamar Blue reagent can be read on a spectrophotometer. Finally, results are analyzed by plotting fluorescence intensity (or absorbance) versus compound concentration.

Materials required but not provided

Mammalian or bacterial cells in appropriate medium Appropriate 96- or 384-well plates
Optional: 3% SDS in phosphate buffered saline (PBS), pH 7.4

Preparing Cells

Mammalian Cells—Adherent: Plate mammalian cells in a cell culture flask or dish, and allow cells to adhere and grow for approximately 4–24 hours at 37°C and 5% CO_2 before proceeding with the assay.

Mammalian Cells—Suspension: Plate mammalian cells in a cell culture flask or dish, and use cells immediately for the assay or allow cells to grow for up to 24 hours at 37°C and 5% CO_2 before proceeding with the assay.

Bacterial Cells: For details, see references 2 and 3. Notes Alamar Blue reagent is stable to multiple freeze/thaw cycles and its activity is not affected if the reagent is frozen.

General Guidelines

Cell types assayed with AlamarBlue reagent include mammalian, bacterial (including biofilms), plant, and fish cells. More specifically AlamarBlue reagent has been tested on hepatocytes, such as HepG2 cells, as well as cells of primary origin.

Be sure to include appropriate assay controls. To minimize experimental errors, we recommend making measurements from a minimum of 4–8 replicates of experimental and no-cell control samples.

You may need to determine the plating density and incubation time for the AlamarBlue assay for each cell type and use conditions such that the assay is in the linear range.

If you plan to use longer incubation time (overnight), be sure to maintain sterile conditions during reagent addition and incubation to avoid microbial contaminants. Contaminated cultures will yield erroneous results as microbial contaminants also reduce AlamarBlue reagent.

Fetal bovine serum (FBS) and bovine serum albumin (BSA) cause some quenching of fluorescence. We recommend using the same serum concentration in controls to account for this quenching. Other media components, such as phenol red do not interfere with the assay.

Protocol

Optional: Treat cells with the test compound 24–72 hours prior to performing the AlamarBlue cytotoxicity assay.Add 1/10th volume of AlamarBlue reagent directly to cells in culture medium.

Adherent Cells

Plate cells in 96-well tissue culture plates in 100 uL/well. For a standard curve, plate a series of cell dilutions in the range of 40-20,000 cells per well for adherent cells,

Suspended Cells

Use 2,000 to 500,000 cells per well for suspension cells. For fluorescence-based detection, include a well with 100 uL of cell culture medium without cells to use as a background control

After cells have reached the desired density, **add 10 uL Alamar Blue solution to the medium in each well (100 ul) , and mix thoroughly.**

Incubate for 1 to 4 hours at 37°C in a cell culture incubator, **protected from direct light**.

Note: Sensitivity of detection increases with longer incubation times. For samples with fewer cells, use longer incubation times of up to24 hours.

Record results using fluorescence or absorbance as follows:

Fluorescence: Read fluorescence using a fluorescence excitation wavelength of 540–570 nm (peak excitation is 570 nm). Readfluorescence emission at 580–610 nm (peak emission is 585 nm).

Absorbance: Monitor the absorbance of AlamarBlue at 570 nm, using 600 nm as a reference wavelength (normalized to the 600 nmvalue).

Note: Fluorescence mode measurements are more sensitive. When fluorescence instrumentation is unavailable, monitor the absorbance of AlamarBlue reagent. Assay plates or tubes can be wrapped in foil, stored at 4°C, and read within 1–3 days without affecting the fluorescence or absorbance values.

Optional: Add 50 µL 3% SDS directly to 100 µL of cells in AlamarBlue reagent to stop the reaction.

References

1. Invest Ophthalmol Vis Sci 38, 1929 (1997); 2. Infect Immun 65, 3193 (1997); 3. J Antimicrob Chemother 57, 1100 (2006) ; 4. Phytochem Anal 12, 340 (2001) ; 5. Anal Biochem 344, 76 (2005)