Resazurin Cell Viability Assay Kit

Catalog Number: 30025-1 (2500 assays)
30025-2 (10,000 assays)

Revised: February 4, 2011

Description

Resazurin Fluorometric Cell Viability Assay Kit offers a simple, rapid, reliable, sensitive, safe and cost-effective measurement of cell viability. This kit performs at least as well as other commercial resazurin-based cell proliferation assay kits with the trademark name AlamarBlue®*. Resazurin 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 [1-3]. 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.

Resazurin assay is as sensitive as [^{3}H] thymidine assay for detecting cell proliferation [1]. Depending on the cell types, Resazurin can detect as few as 40 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.

Kit Component

30025-1 (2500 assays)	**30025-2** (10,000 assays)	
25mL	100mL	Resazurin solution (sterile)

Storage and Handling

Upon receipt, the kit should be stored at 4°C and protected from light. Stored properly, the kit components should remain stable for at least 6 months.

Experimental Protocol

Standard Curve

1. Plate cells in 100μL medium into 96-well tissue culture plates by conducting cell number titration in the range of 40 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 resazurin solution 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.

Resazurin Fluorometric Cell Viability Assay

1. Plate cells into 96-well tissue culture plates using optimal cell concentration.
2. Carry out your experiment by adding agents of your interest into appropriate well and incubate with cells for a certain period of time.
3. Add 10μL resazurin solution into medium and incubate cells for at least 1 hour and up to 24 hours at 37°C.
4. 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.
5. 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.

References

1. Ahmed SA, Gogal RM Jr, Walsh JE. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay. J Immunol Methods. 1994 Apr 15;170(2):211-24.
2. Shahan TA, Siegel PD, Sorenson WG, Kuschner WG, Lewis DM. A sensitive new bioassay for tumor necrosis factor. J Immunol Methods. 1994 Oct 14;175(2):181-7.
3. Nociari MM, Shalev A, Benias P, Russo C. A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity. J Immunol Methods. 1998 Apr 15;213(2):157-67.

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