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Product Information

Resazurin Cell Viability Assay Kit

Kit Contents

30025-1 (2500 assays): 25 mL resazurin solution
30025-2 (10,000 assays): 100 mL resazurin solution

Storage and Handling

Store at 4°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Spectral Properties

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

Product Description

Resazurin Fluorometric Cell Viability Assay Kit offers a simple, rapid, reliable, sensitive, safe and cost-effective measurement of cell viability. The assay is homogenous and requires no cell lysis or washing. Biotium's resazurin assay performs at least as well as other commercial resazurin-based cell proliferation assay kits with the trademark name AlamarBlue®.

Cell growth creates a reduced environment while inhibition of growth maintains an oxidized environment. In response to chemical reduction of growth medium resulting from cell growth, resazurin (which is purple and non-fluorescent) is reduced to form the red fluorescent dye resorufin (1-3). Reduction of resazurin can be monitored by measuring fluorescence or absorbance. The fluorescent and colorimetric signal generated from the assay is proportional to the number of living cells in the sample. The resazurin assay is as sensitive as [^{3}H] thymidine assay for detecting cell proliferation (1). Depending on the cell type, the resazurin assay can be used to detect as few as 40 cells with reproducible and sensitive signal.

References

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

Assay Protocol

Note: Resorufin can be further reduced to hydroresorufin (colorless and non-fluorescent). At higher cell densities or with prolonged development times, the assay signal can initially increase, then decrease after all resazurin is converted into resorufin, which then begins to be further reduced to hydroresorufin. Therefore, it is important to conduct a cell number titration (standard curve) to identify the optimal plating density and development time that generates signal that increases proportionally with cell number.

1. 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, and 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.
2. After cells have reached the desired density, add 10 uL resazurin solution to the medium in each well, and mix thoroughly.
3. Incubate the plate for between 1 hour and 24 hours at 37°C.

   Note: Signal from the same plate can be read at multiple time points to determine the optimal incubation time for your cell type and density.
4. For colorimetric detection, measure absorbance at 570 nm and 600 nm using an absorbance microplate reader. For fluorescence-based detection, measure fluorescence with excitation/emission at 570/585 nm using a fluorescence microplate reader.

   Note: Fluorescence-based detection is more sensitive and has broader dynamic range than colorimetric detection.

   Note: The excitation and emission spectra of resorufin are fairly broad, excitation filters between 530–570 nm and emission filters between 580-620 nm can be used.
5. For the colorimetric detection method, subtract background absorbance at 600 nm from resorufin absorbance at 570 nm. For fluorescence-based detection, subtract fluorescence at 585 nm from the background control (culture medium without cells) from each cell sample.
6. Plot cell plating density vs. background-subtracted absorbance or fluorescence for your cell number titration to determine the optimal assay conditions for your cell line.

Related Products

Cat. no.	Product
30026	Calcein AM Cell Viability Assay Kit
30006	MTT Cell Viability Assay Kit
30007	XTT Cell Viability Assay Kit
30020	ATP-Glo™ Bioluminometric Cell Viability Assay Kit
30068	ViaFluor™ 405-SE Cell Proliferation Kit
30066	Apoptotic, Necrotic, and Healthy Cells Quantitation Kit Plus
30060	CF™488A Annexin V and 7-AAD Apoptosis Kit
30061	CF™488A Annexin V and PI Apoptosis Kit
32002-32009	Live-or-Dye™ Fixable Viability Staining Kits (choose from 8 fluorescent dye colors)
30029	NucView™ 488 Caspase-3 Assay Kit for live cells
30067	Dual Apoptosis Assay with NucView™ 488 and CF™594 Annexin V
30072	NucView™ 488 and RedDot™ 2 Apoptosis and Necrosis Kit
10405	NucView™ 405 Caspase-3 Substrate
30019	MCB Glutathione Detection Kit

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