

Fluorometric Cell Viability Kit I (Resazurin)



Instruction Manual

Product Name	Product Description	Size	Catalog Number
Fluorometric Cell Viability Kit I	Fluorometric Cell Viability Kit I (Resazurin)	5 ml (500 assays)	PK-CA707-30025-0
		25 ml (2500 assays)	PK-CA707-30025-1
		100 ml (10000 assays)	PK-CA707-30025-2

Introduction

The PromoKine Fluorometric Cell Viability Assay Kit I (Resazurin) offers a simple, rapid, reliable and sensitive measurement of cell viability. The assay is homogenous and requires no cell lysis or washing. Moreover, this resazurin-based assay is very safe and cost-effective and allows to measure the proliferation of animal, bacteria, plant, and fungi cells and the cytotoxicity of various agents quantitatively. PromoKine's resazurin assay performs at least as well as other commercial resazurin-based cell viability/proliferation assay kits with the trademark name AlamarBlue®.

Resazurin a non-toxic, cell-permeable compound utilizes the reducing power of living cells to detect cell viability by converting from a non-fluorescent blue dye to the highly fluorescent red dye resorufin in response to changes of the reducing environment within the cytosol of the cell⁽¹⁻³⁾. Healthy, living cells and continued cell growth create and maintain a reduced environment while inhibition of growth maintains an oxidized environment. Chemical reduction related to growth/cell metabolism converts resazurin (which is purple and non-fluorescent) to its reduced form resorufin (red color, fluorescent). The fluorescent signal of the resorufin is monitored using 530-570 nm excitation wavelength and 585-590 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. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and color of the cell culture media surrounding the cells. Thus, 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 the [³H] thymidine assay for detecting cell viability/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 Contents

Components:	PK-CA707-30025-0	PK-CA707-30025-1	PK-CA707-30025-2
	500 assays	2,500 assays	10,000 assays
Resazurin Solution (sterile)	5 ml	25 ml	100 ml

Storage and Stability

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.

Cell Proliferation Assay Procedures

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 µl/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 µl of cell culture medium without cells to use as a background control.
2. After cells have reached the desired density, add 10 µl 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.

Intended Use

For *in vitro* research use only. Not for diagnostic or therapeutic procedures.

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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