#11884 Store at –20°C

Resazurin Cell Viability Kit

Cell Signaling TECHNOLOGY®

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Description: The Resazurin Cell Viability Kit is a fluorescent assay that detects cellular metabolic activity. The blue nonfluorescent resazurin reagent is reduced to highly fluorescent resorufin by dehydrogenase enzymes in metabolically active cells. This conversion only occurs in viable cells and thus, the amount of resorufin produced is proportional to the number of viable cells in the sample. The resorufin formed in the assay can be quantified by measuring the relative fluorescence units (RFU) using a fluorometer (Ex=530-570 nm, Em=590-620 nm).

Background: Cell viability assays are widely used in drug discovery for the study of growth factors, cytokines, and cytotoxic agents. High throughput screening, in both early drug discovery compound screening and subsequent drug safety and toxicity studies, require reliable, sensitive, and simple assays with the ability to analyze a large number of samples. Colorimetric cell viability assays using tetrazolium salt, such as MTT, XTT, and WST-1, were developed based on live cell reduction of tetrazolium salt into highly colored formazan compounds (1,2). Similarly, resazurin (blue and nonfluorescent) can be reduced to resorufin (pink and highly fluorescent) in live cells and is therefore used to assess mammalian cell toxicity, viability, migration, and invasion (3-6). Similar to the XTT Cell Viability Kit #9095, the Resazurin Cell Viability Kit does not require radioactive materials, cell fixation, or cell permeabilization, and cells used in this assay may be used for further analysis.

Specificity/Sensitivity: The Resazurin Cell Viability Kit detects resorufin produced from resazurin conversion by metabolic enzymes in live cells. This kit is expected to work in most cell lines. For most experiments, 0.02-2x10^5 cells/well should be sufficient, but this can vary depending on the cell type and incubation time. For best results, a cell number titration and incubation time course (as shown in Figure 1) is recommended.

Note: Microbial contaminants will reduce resazurin to resorufin, yielding false positive results. Autofluorescent compounds may interfere with this assay.

Background References:

(1) Scudiero, D.A. et al. (1988) *Cancer Res* 48, 4827-33.

(2) Roehm, N.W. et al. (1991) *J Immunol Methods* 142, 257-65.

(3) Nakayama, G.R. et al. (1997) *J Immunol Methods* 204, 205-8.

(4) Al-Nasiry, S. et al. (2007) *Hum Reprod* 22, 1304-9.

(5) Anoopkumar-Dukie, S. et al. (2005) *Br J Radiol* 78, 945-7.

(6) O'Brien, J. et al. (2000) *Eur J Biochem* 267, 5421-6.

Products Included	Quantity	Solution Color
Resazurin solution	25 ml	Blue

Note: Resazurin solution should be stored at –20°C in the dark for long term storage. It can be stored at 4°C in the dark for up to 24 months.

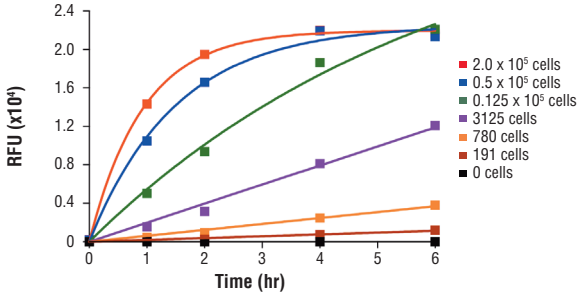

Figure 1. HeLa cells were seeded at varying density in a 96-well plate and incubated overnight. The Resazurin solution (10% of cell culture volume) was added to the plate and relative fluorescent units were measured at 0, 1, 2, 4, and 6 hr.

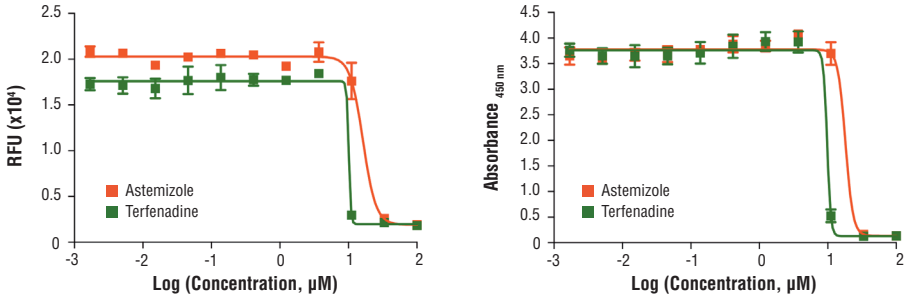

Figure 2. Jurkat cells were seeded at 1x10^5 cells/well in a 96-well plate and then treated overnight with various concentrations of astemizole or terfenadine. Cytotoxicity was measured using the Resazurin Cell Viability Kit (left), followed by BrdU Cell Proliferation Assay Kit #6813 (right).

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Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: H—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#11884

Resazurin Cell Viability Assay Protocol

A.

1. Thaw out Resazurin solution (if kept frozen) and warm it to 37°C to ensure all components are completely in solution.
2. Plate cells in 96-well plate (black plate with clear bottom). Typical seed cell number is 0.02-2x10^5 cells/well depending on cell growth rate. Cell number titration is recommended to determine the optimal cell seeding density.
3. Incubate cells with compound of interest for desired period of time (1-72 hr). Make sure all the wells contain the same volume of medium.
4. Add Resazurin solution to plate (10% of the initial volume in the well). For example, for plates containing 100 µl medium/well, add 10 µl resazurin solution to each well.
5. Incubate the plate for 1-6 hr in standard culture conditions. Incubation time depends on cell type and cell number. The plate can be read multiple times to determine the optimal time point.
6. Measure the relative fluorescent units (RFU) using a plate reader. Ex=530-570 nm, Em=590-620 nm.

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