

Resazurin Cell Viability Assay

One-step Sensitive Quantitation for Cell Viability

Catalog Number	Reagent Volume	Assay Size
TBS2001-2K	20 mL	2000 assays
TBS2001-5K	50 mL	5000 assays
TBS2001-10K	100 mL	10000 assays

DESCRIPTION

Resazurin Cell Viability Assay provides a homogeneous, fluorescent method for monitoring cell viability. It uses the indicator dye resazurin to measure the metabolic capacity of cells - an indicator of cell viability. Viable cells retain the ability to reduce resazurin into resorufin, which is highly fluorescent. Nonviable cells rapidly lose metabolic capacity, do not reduce the indicator dye, and thus do not generate a fluorescent signal. The homogeneous assay procedure involves adding the single reagent directly to cells cultured in serum-supplemented medium. After an incubation step, data are recorded using either a plate-reading fluorometer (570_{Ex}/590_{Em}) or spectrophotometer (Resazurin 605nm /Resorufin 573nm). However, fluorescence is the preferred method because it is more sensitive and involves fewer data calculations. Therefore, the fluorescence intensity observed in this assay is a true measure of the viable cells. The reagent is compatible with all culture media and with all liquid handling systems for high-throughput screening applications in 96-well and 384-well plates.

APPLICATIONS

Direct Assays: Cell proliferation, cytotoxicity, and apoptosis.

KEY FEATURES

- **Sensitive and accurate:** As low as 100 cells can be accurately quantified. There is a linear relationship between cell number and fluorescence/ absorbance.
- **Save Your Time:** The homogeneous add-incubate-measure format reduces the number of handling steps.
- **Robust and amenable to HTS:** Can be readily automated on HTS liquid handling systems.

KIT CONTENTS

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STORAGE AND HANDLING

Store the Reagent at -20°C, and protect from light. For frequent use, the product may be stored tightly capped at 4°C or at ambient temperature (22-25°C) for 6-8 weeks. Shelf life: 12 months.

PROTOCOL

1. Culture cells in black (clear for absorbance) 96-well or 384-well tissue culture plates with 100 µL or 50 µL medium. The number of cells can vary from 100 to 80,000 per well. Set up triplicate-wells without cells to serve as the **blank control** to determine background fluorescence that may be present. Set up triplicate wells with untreated cells to serve as a **vehicle control**. Add the same solvent used to deliver the test compounds to the vehicle control wells. Set up triplicate wells containing cells treated with a compound known to be toxic to the cells used in your model system.

2. Compound treatment cells for the desired timer period (2-12hr).
3. Equilibrate the Reagent to room temperature. Add 10 µL of the reagent per well (5 µL for 384-well format). Tap plate to mix. Incubate for 1 to 4 hours at 37°C.
4. Measure fluorescent intensity for each well on a fluorescence plate reader. Options for fluorescence filter sets include 530-570nm for excitation and 580-620nm for fluorescence emission. If absorbance measurements are used to record data, we recommend taking readings at 570nm and using 600nm as a reference wavelength.

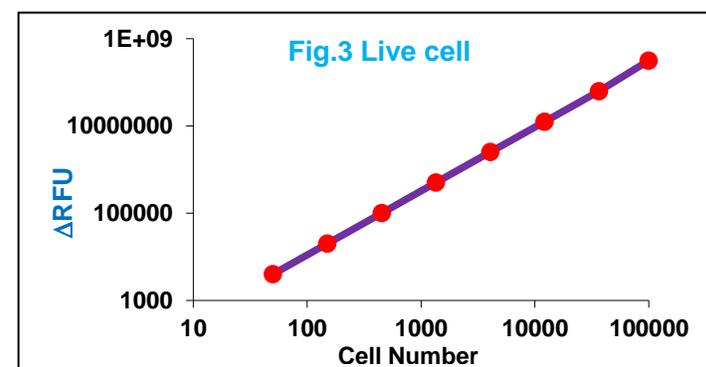
CALCULATION OF RESULTS

Fluorescence Data Subtract the average of fluorescence values of the culture medium background from all fluorescence values of experimental wells. Plot fluorescence vs. concentration of test compound. **Absorbance Data** Subtract the average of absorbance 600nm values of the culture medium background from all absorbance 570nm values of experimental wells. Plot 570-600nm absorbance vs. concentration of test compound.

For cell proliferation or cytotoxicity assays, the activity of a test compound can be calculated as percent change in cell number as follows:

$$\text{Activity (\%)} \text{ or Cell viability (\%)} = 100 \times (F_{\text{sample}} - F_0) / (F_{\text{ctrl}} - F_0)$$

Where F_{sample} and F_{ctrl} are the average fluorescence intensities in the presence and absence (vehicle control) of the test compound, F_0 is the averaged blank control fluorescence intensity.



RELATED PRODUCTS

- LDH Cytotoxicity Assay (TBS2002)
- ATP Colorimetric/Fluorometric Assay (TBS2010)
- ADP Colorimetric/Fluorometric Assay Kit (#TBS2020)
- Amplex Red Hydrogen Peroxide Assay Kit (TBS2066)
- AmplexRed HRP-System (TBS5026)
- Tryptase Activity Assay (TBS2101)
- β-Hexosaminidase Activity Assay (TBS2105)
- Cytochrome C Oxidase Activity Assay (TBS2115)

Research Use only