Resazurin Cell Viability Assay Kit
(2500 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-77101-2500

www.zellx.de

Application:

Detection of Cell Viability, Proliferation & cytotoxicity

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

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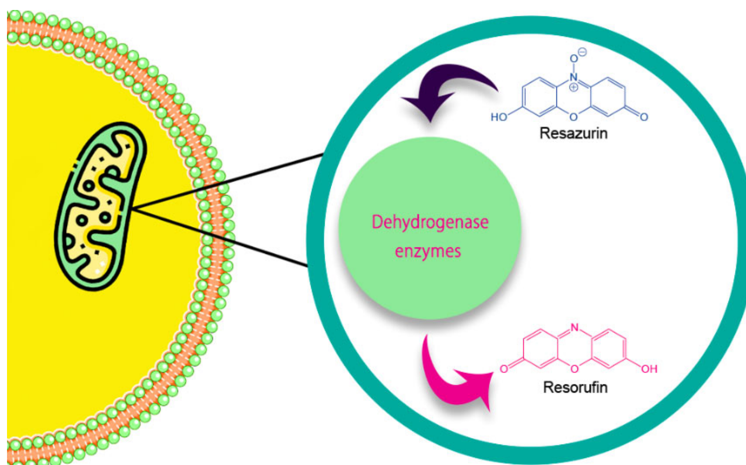
Background

Resazurin (7-hydroxy-10-oxidophenoxazin-10-ium-3-one, sodium) is a blue non-fluorescent dye used as a cell viability and proliferation indicator for bacteria, yeast and mammalian cells. Dehydrogenase enzymes in the mitochondrial respiratory chain of the metabolically viable cells irreversibly reduce the oxidized form (blue) of the dye to resorufin (a highly pink-fluorescent product), which exhibits an emission around 590 nm. Resazurin was originally used to test bacterial and yeast contamination in milk and other biological fluids.



The Resazurin Cell Viability assay is a fluorescent/colorimetric assay that measures cellular metabolic activity based on conversion of the resazurin reagent to resorufin. As the conversion only occurs in metabolically active cells, the amount of The fluorescent or colorimetric signal generated due to resorufin production is proportional to the number of viable cells in the sample. The reduction of resazurin to resorufin in the assay can be quantified either by measuring the fluorescent intensity at 590 nm (590-620 nm) with the excitation at 550 nm (530-570 nm), or the optical density at 570 nm.

Intended use

Determination of cell viability, cell proliferation and cytotoxicity.

Materials supplied in the Kit

Component	*Quantity*
Resazurin Reagent	25 mL

Storage instruction

All reagents should be stored at -20° C, protected from light, until the expiration date of the kit.

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Materials required but not supplied

Precision pipettes and disposable pipette tips

Sterile clear 96 well plate

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- Equilibrate all kit components to room temperature (RT) before use.
- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Sample preparation

i. One to three days before the experiment, cultivate cells (0.5 - 2 x 10^4 cells/well) into a 96-well plate containing 100 µL/well of cell culture medium. The appropriate time of incubation may vary depending on the cell types and the desired chemical or physical treatments and must be evaluated by the end user.

ii. Each plate must be included at least two wells as background control which contain 10 µL of the Resazurin Reagent + 100 µL complete media.

- We strongly recommend to determine the optimal cell number and incubation time for your specific cells before performing a large number of Resazurin assays.

Assay Procedure

1. **Optional**: treat the cells with compounds of interest dissolves in an appropriate solvent for desired time period. In the case of treatment include control wells for the solvent, the cells must be treated with the same solvent without compounds of interest.
2. warm the Resazurin Reagent to 37°C to ensure all components are completely in solution
3. Add 10 µL of the Resazurin Reagent to each well. Mix gently for one minute.

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4. Incubate the cells for 3-5 hours (adherent cells & suspension cells) at 37°C in a CO_2 incubator. **(Incubation times may vary depending on the metabolic rates of the cells being tested and can be prolonged up to 24 hours).**
5. Read the optical density at 570 nm (for colorimetric measurement), or read the fluorescent intensity at 590 nm with the excitation at 550 nm (for fluorometric measurment).

Calculations

i. Average the duplicate optical density (OD)/ Fluorescent Unit (FLU) readings for each sample.
ii. Subtract the background absorbance from the signal absorbance to obtain normalized absorbance values.

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