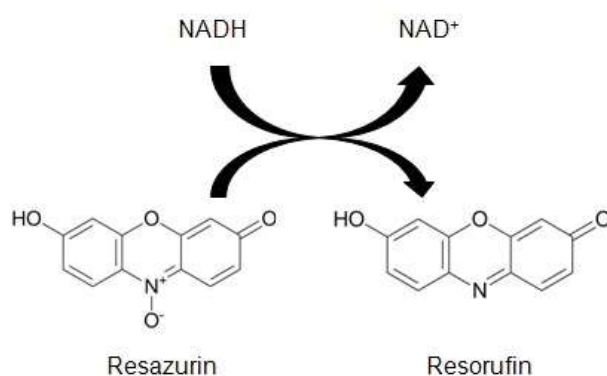


Resazurin Reduction Assay

The Resazurin Cell Viability Assay (Alamar blue) is a fluorescent assay that detects cellular metabolic activity. The blue non-fluorescent 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 microplate fluorometer (Ex=530-570 nm, Em=590-620 nm).



- **Prepare Master resazurin solution at concentration 4000ug/ml (4mg/ml).**

Dilute 0.1 gr Resazurin to 25 ml sterilized water

- **Prepare working resazurin solution diluting master solution at final concentration 60 µg/ml using complete cell culture medium**
 - Add 20 ul of working resazurin solution to the wells already containing 100ul cells (final resazurin concentration 10 µg/ml) 2 hours ± 10 minutes before the end of the exposure.
 - Plates are closed and shaken for 10 sec
 - Plates are visually inspected to make sure that resazurin solution was added and mixed well in every well
 - The exposure was continued for 2-hours ± 10 minutes at 37.0 ± 1 °C in a humidified incubator with 5.0 ± 0.5% CO₂.
 - After the incubation, plates are shaken for another 10 seconds and visually inspected for the presence of air bubbles which are carefully removed to avoid interference with optical readouts
 - The fluorescence is determined, using a plate reader (Varioscan LUX, Thermo Fisher Scientific) adjusted accordingly.

The measurement of fluorescence is performed at 530/25 nm excitation wavelength and 590/35 nm emission wavelengths.