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TechnicalService

IN VITRO TOXICOLOGY ASSAY KIT
Resazurin Based

Stock No. TOX-8
Store at 2-8°C

This kit is designed for fluorometrically or spectrophotometrically determining cell number as a function of metabolic activity using the dye resazurin.

IT IS RECOMMENDED THAT THE ENTIRE PROTOCOL BE REVIEWED BEFORE STARTING THE ASSAY.

Product Description

Traditionally, the toxic effects of unknown compounds have been measured in vitro by counting viable cells after staining with a vital dye. Alternative methods include the measurement of DNA synthesis by radioisotope incorporation, cell counting by automated counters and other methods which rely on dyes and cellular activity. The resazurin system measures the metabolic activity of living cells.

The resazurin method is simple, accurate and reproducible. The key component is the oxidoreduction indicator dye resazurin. Solutions of resazurin, prepared in balanced salt solutions without phenol red, are dark blue in color. Bioreduction of the dye by viable cells reduces the amount of its oxidized form [blue] and concomitantly increases the amount of its fluorescent intermediate [red], indicating the degree of cytotoxicity caused by the test material. The amount of dye conversion in solution is measured fluorometrically or spectrophotometrically.

REAGENT
For Research Use Only.
Not for Use in Diagnostic Procedures.

Kit Components

Catalog No.	Item	Quantity
R-6892	Resazurin Solution	25 ml

Procedure

The resazurin method of monitoring *in vitro* cytotoxicity is well suited for use with multiwell plates. For best results, use cells in the log phase of growth and a final cell density less than 10^6 cells/cm^2. Each test should include a blank containing complete medium without cells.

NOTE: Bacteria, mycoplasma and other microbial contaminants may also reduce the resazurin dye. Cultures containing microorganisms should not be assayed using this method.

1. Remove cultures from incubator into laminar flow hood or other sterile work area.
2. Add resazurin dye solution in an amount equal to 10% of the culture medium volume.
3. Return cultures to incubator for 2-4 hours depending on cell type and maximum cell density. (An incubation period of 2 hours is generally adequate but may be lengthened for low cell densities or cells with lower metabolic activity.) Incubation times should be consistent when making comparisons.
4. Gentle mixing in a gyratory shaker will enhance distribution of the dye.
5. Samples can be measured spectrophotometrically by monitoring the decrease in absorbance at a wavelength of 600 nm. Measure the absorbance of multiwell plates at a reference wavelength of 690 nm and subtract from the 600 nm measurement. Alternatively, samples can be measured fluorometrically by monitoring the increase in fluorescence at a wavelength of 590 nm using a excitation wavelength of 560 nm. **NOTE:** Fluorometric detection is much more sensitive than spectrophotometric detection and the number of cells used in the assay should be

reduced proportionally.

6. Tests performed in multiwell plates can be read using an appropriate type of plate reader or the contents of individual wells may be transferred to appropriate size cuvets for fluorometric or spectrophotometric measurement.

Possible Sources of Error

1. Microbial contamination will contribute to the reduction of the resazurin dye yielding erroneous results.
2. Uneven evaporation of culture fluid in wells of multiwell plates may cause erroneous results.
3. Media and salt solutions with phenol red can be used but will contribute to higher background absorbance and can decrease sensitivity.

References

1. Dutka, B.J., N. Nyholm and J Petersen. [1983] Comparison of several microbiological toxicity screening tests. Water Research 17:1363-1367.
2. King, E.F. [1984] A comparative study of methods for assessing the toxicity to bacteria of single chemicals and mixtures. In: Toxicity Screening Procedures Using Bacterial Systems, D. Liu and B.J. Dutka eds., Decker, New York. pp. 175-194.
3. Liu, D. [1981] A rapid biochemical test for measuring chemical toxicity. Bull. Environmental Contamination Toxicology 26:145-149.
4. Strotmann, U.J., B. Butz and W-R Bias. [1993] The dehydrogenase assay with resazurin: Practical performance as a monitoring system and pH-dependent toxicity of phenolic compounds. Ecotoxicology Environmental Safety 25:79-89.

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