Technical Bulletin

LDH Cytotoxicity Assay Kit

Catalogue number MAK529

Product Description

Lactate dehydrogenase (LDH) is an oxidoreductase which catalyzes the interconversion of lactate and pyruvate. Cytotoxic compounds often compromise cell membrane integrity by inducing apoptosis or necrosis. LDH is a stable cytosolic enzyme that upon membrane damage is released into the cellular environment. Therefore, LDH is often measured to evaluate the presence of tissue or cell damage.

The LDH Cytotoxicity Assay Kit is a simple and robust method to assess cytotoxic effects on cells by measuring the activity of LDH in cell culture supernatant. The assay is based on the reduction of a tetrazolium salt to a formazan dye.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Reagent 20 mL
  Catalogue Number MAK529A
- Tergitol 1 mL
  Catalogue Number MAK529B

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., multichannel pipettor)
- Spectrophotometric multiwell plate reader.
- Clear flat-bottom 96-well tissue culture plates. (Catalogue Number M0812 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at -20 °C.

Preparation Instructions

Equilibrate all components to room temperature prior to use.

Sample Preparation

Recommended to prepare assay in duplicate or triplicate.

The assay can be performed on either adherent cells or cells in suspension. The number of cells can vary with cell type, but a range between 10,000 and 40,000 cells per well for adherent cells and between 40,000 and 160,000 suspension cells will be appropriate for most mammalian cell types.

Note: Medium containing 10% FBS is compatible with the assay. It is not necessary to subculture cells in FBS free medium.

Cells should be in logarithmic growth phase for the assay. Subculture cells 2 days before the experiment. Plate and culture cells (100 µL per well) using a 96-well tissue culture plate. In addition to the test samples, include extra wells of cells without treatment (Control) and wells of cells treated with Tergitol (Total Lysis).

In addition to the test samples, include extra wells of cells without treatment (Control) and wells of cells treated with Tergitol (Total Lysis). Add 10 µL test compounds to sample wells, 10 µL purified water to Control wells, and 10 µL of Tergitol to the Total Lysis control wells. Incubate cells for 10 minutes to overnight at 37 °C.

Procedure

Assay Reaction

Equilibrate reagent to room temperature.

1. Add 160 µL of Reagent per well and incubate at room temperature for 10 minutes.
2. Measure the optical density at 500 nm for each well in an absorbance plate reader.

Note: The suitable absorbance range for the formazan dye is between 490 and 510 nm. 500 nm is recommended for this assay.

Results

1. The cytotoxicity is calculated as the percentage of the maximum LDH release in the Total Lysis wells compared to the Sample Wells:

$$\text{Cytotoxicity} = \frac{OD_{Sample} - OD_{Control}}{OD_{Total\ Lysis} - OD_{Control}} \times 100\ (\%)$$

Where:

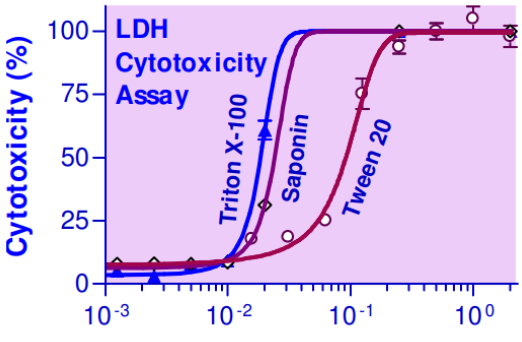
OD_{Sample} = Optical density of the Sample wells.

$OD_{Control}$ = Optical density of the Control wells.

$OD_{Total\ Lysis}$ = Optical density of the Total Lysis wells.

Figure 1.

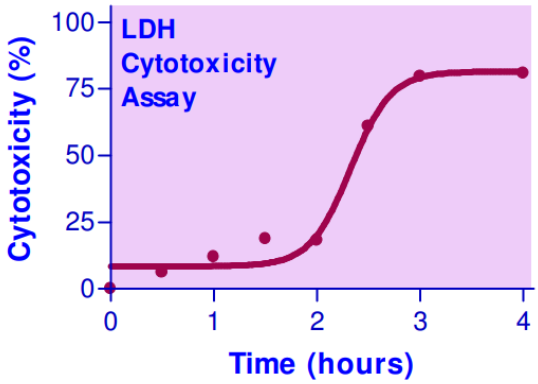
PANC-1 cells were seeded at 20,000 cells per well and allowed to settle overnight. Test compounds (Triton X-100, Saponin, Tween 20) were diluted in complete medium (RPMI1640, 10% FBS) and incubated with cells for 4 hours. Cytotoxicity of test compounds was assayed as increased release of LDH into the culture media.



Dose Response (4 hours)

Figure 2.

PANC-1 cells were seeded at 20,000 cells per well and allowed to settle overnight. 0.025% Triton X-100 was diluted in complete medium, then added to cells at the appropriate time. Cytotoxicity of test compounds was assayed as increased release of LDH into the culture media.



Time Response (0.025% Triton X-100)

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