

LDH Cytotoxicity Assay Kit (Colorimetric)

LS-K240-100 (100 Tests) • Store at -20°C



Introduction

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 colorimetric LDH release assay 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.

Key Features

- Safe. Non-radioactive assay.
- Fast. The whole procedure takes 20 min. Single reagent, "mix-incubate-measure" type assay. High-throughput assay in 96-well plates allows simultaneous processing tens of thousands of samples per day
- Robust and amenable to HTS.

Applications

- Cytotoxicity and Apoptosis: evaluation of toxic compounds, toxins, detergents, environmental pollutants, etc.
- Drug Discovery: high-throughput screen for drug toxicity.

Components

Component	K240-100
	100 Tests
Reagent	20 mL
Triton X-100 (20%)	1 mL

Materials Not Supplied

Pipetting devices and accessories, clear flat-bottom 96-well tissue culture plates (e.g. Sigma cat# M0812), microplate reader.

Storage

The kit is shipped at room temperature. Store kit at -20°C upon receiving. Shelf life: 12 months after receipt.

Assay Procedure

Sample Preparation

Cells should be in logarithmic growth phase for the assay. Subculture cells 2 days before the experiment. Medium containing 10% FBS is compatible with the assay: it is not necessary to subculture cells in FBS free medium. Plate and culture cells (100 µL per well) in a 96-well tissue culture plate. It is recommended that assays be run in duplicate or triplicate. Assays 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.

FOR RESEARCH USE ONLY! Not for use in humans.

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In addition to the test samples, one must include extra wells of cells without treatment (Control) and wells of cells treated with Triton X-100 (Total Lysis).

Add 10 µL test compounds to sample wells, 10 µL dH₂O to Control wells, and 10 µL 20% Triton X-100 to the Total Lysis control wells, and incubate cells for 10 min to overnight at 37°C.

Procedure

1. Equilibrate reagent to room temperature. Add 160 µL of Reagent per well and incubate at room temperature for 10 minutes.
2. Measure OD_{500nm} for each well in an absorbance plate reader. The suitable absorbance range for the formazan dye is between 490 and 510 nm. We recommend reading at 500 nm.

Calculations

Cytotoxicity is calculated as the percentage of the maximum LDH release in the Total Lysis wells in the Sample wells, as follows:

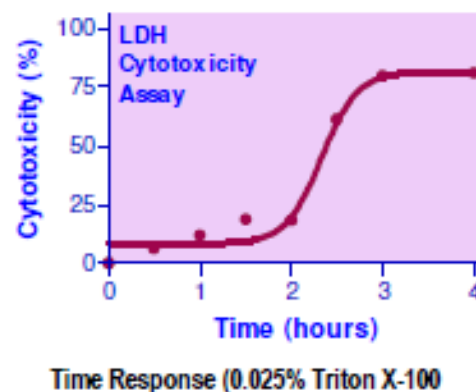
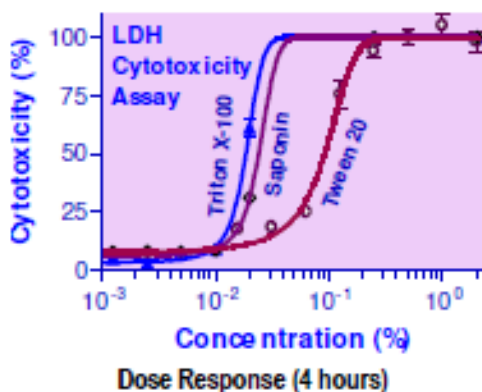
$$\text{Cytotoxicity} = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Total Lysis}} - \text{OD}_{\text{Control}}} \times 100 (\%)$$

Where OD_{Sample}, OD_{Control} and OD_{Total Lysis} are absorbance values of the sample, the no treatment control and the Triton X-100 treated cells respectively

Sample Data

Dose Response: 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.

Time Response: 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.



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