QuantiChrom™ LDH Cytotoxicity Assay Kit (CQLD-100)

Quantitative Colorimetric Assay of LDH Released in Cell Culture Medium

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 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 the tetrazolium salt MTT to a formazan dye which exhibits an absorption maximum at 565 nm.

KEY FEATURES

Safe. Non-radioactive assay (cf. chromium release assay).

Fast. High-throughput assay using 96-well plates allows simultaneous processing tens of thousands of samples per day.

Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash steps are involved.

Robust and amenable to HTS. Can be readily automated with HTS liquid handling systems.

APPLICATIONS

Cytotoxicity and Apoptosis: evaluation of toxic compounds, toxins, detergents, environmental pollutants etc.

Drug Discovery: high-throughput screen for drug toxicity.

KIT CONTENTS (100 Tests in 96-Well Plate)

Substrate Buffer:	20 mL	**PMS Reagent:**	1.5 mL
NAD Solution:	1 mL	**10% Triton X-100:**	1 mL
MTT Reagent:	1.5 mL		

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

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.

1. Plate and culture cells (200 µL per well) in a 96-well tissue culture plate. Assays can be performed on either adherent cells or cells in suspension. The number of cells can varies with cell type but a range between 10,000 and 40,000 cells per well for large adherent cells and between 40,000 and 160,000 suspension cells will be appropriate for most mammalian cell types. In addition to the test samples, one must include control wells of culture medium containing no cells (Medium) and cells treated with Triton X-100 (Total Lysis).
2. Add 20 µL test compounds to samples wells and 20 µL Triton X-100 to the Total Lysis control wells, and incubate cells for four hours or desired period of time at 37°C. It is recommended that assays be run in duplicate or triplicate.
3. After the incubation remove 20 µL cell-free supernatant from each well and transfer to a clear flat-bottom 96-well plate. If necessary, briefly spin the plate for 5 min at 300 x g to pellet the cells before removing the supernatant.
4. Reagent Preparation: Equilibrate reagents to room temperature. The Working Reagent is prepared by mixing for each 96-well assay, 14 µL MTT Solution, 8 µL NAD Solution, 8 µL PMS Solution and 170 µL Substrate Buffer. Fresh reconstitution is recommended.

   Add 180 µL of Working reagent per well and incubate at room temperature for 30 minutes.
5. Measure OD_{565nm} for each well in an absorbance plate reader. The suitable absorbance range for the formazan dye is between 520 and 590 nm with the maximum absorbance at 565 nm.

CALCULATION

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

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

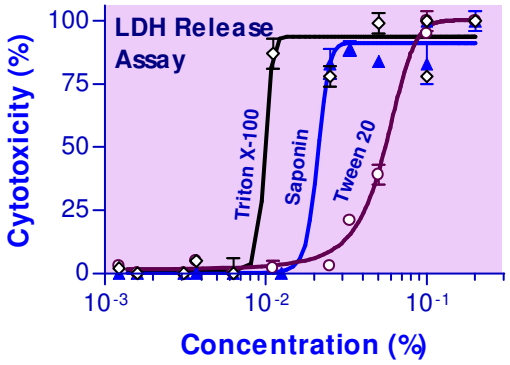
Where OD_{Sample}, OD_{Medium} and $OD_{Total\ Lysis}$ are absorbance values of the sample, the no cells control and the Triton X-100 treated control.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories, clear flat-bottom 96-well plates, microplate reader.

EXAMPLE

HL-60 leukemic cells were seeded at 120,000 cells per well immediately prior to the assay. Test compounds (Triton X-100, Saponin and 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.



LITERATURE

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3. Kawai et al. (1992). Additive effects of antitumor drugs and lymphokine-activated killer cell cytotoxic activity in tumor cell killing determined by lactate-dehydrogenase-release assay. Cancer Immunol Immunother 35(4):225-229.