# Technical Manual

# ChromaDazzle LDH Cytotoxicity Assay Kit

# Catalogue Code: BA0001

# Pack Size: 100 assays

# Research Use Only

## 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 a tetrazolium salt to a formazan dye.

## KEY FEATURES

**Safe**. Non-radioactive assay.

**Fast**. A 10 min, single reagent, "mix-incubate-measure" type assay. High-throughput assay using 96-well plates allows simultaneous processing tens of thousands of samples per day.

**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)

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|---|---|---|---|
| **Reagent:** | 20 mL | **Triton X-100:** | 1 mL 20% |

**Storage conditions**. The kit is shipped at room temperature. Store kit at 2-8°C upon receiving. Shelf life: 12 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. 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.

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 $dH_2O$ 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.

**Assay**:

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.

## CALCULATION

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

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

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.

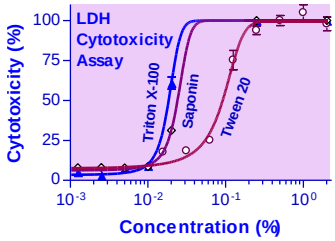
## MATERIALS REQUIRED, BUT NOT PROVIDED

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

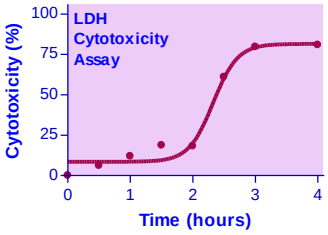
## EXAMPLE

*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.




**Dose Response (4 hours)**




**Time Response (0.025% Triton X-100**

## LITERATURE

1. Korzeniewski C. and Callewaert DM. (1983). An enzyme-release assay for natural cytotoxicity. J Immunol Methods 64(3): 313-320.
2. Weidmann E. et al. (1995). Lactate dehydrogenase-release assay: A reliable, nonradioactive technique for analysis of cytotoxic lymphocyte-mediated lytic activity against blasts from acute myelocytic leukemia. Ann Hematol 70(3): 153-158.
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.

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