Product Manual

CytoSelect™ LDH Cytotoxicity Assay Kit

Catalog Number

CBA-241	960 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

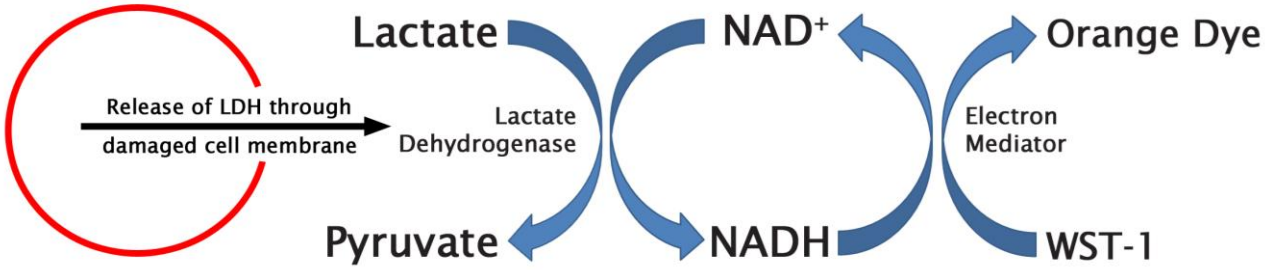
CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

The measurement and monitoring of cell cytotoxicity is an essential technique in any laboratory focused on cell-based research. This skill allows for the optimization of cell culture conditions. More importantly, the cytotoxic nature of anticancer compounds in toxicology testing, the toxicity of therapeutic chemicals in drug screening, and cell-mediated cytotoxicity can all be assessed through this assay-based approach.

Cell cytotoxicity characteristics include loss of cellular metabolic activity or cell membrane integrity. One method for measuring metabolic activity is to incubate the cells with a tetrazolium salt such as MTT, which is cleaved into a colored formazan product by metabolically active cells. Similarly, the green fluorescent dye Calcein AM can measure intracellular esterase activity in proliferating live cells, while dyes such as trypan blue or propidium iodide can enter and stain cells that have lost membrane integrity.

Cell Biolabs' CytoSelect™ LDH Cytotoxicity Assay Kit provides a colorimetric format for measuring and monitoring cell cytotoxicity. The kit contains sufficient reagents for the evaluation of 960 assays in 96-well plates. Cells can be plated and then treated with compounds or agents that affect cell viability. Upon cell death, lactate dehydrogenase (LDH), a soluble enzyme found in the cytoplasm, is released into the growth media. The growth media is then transferred to another plate and the released LDH is then detected with a cytotoxicity reagent. In the presence of lactate substrate (included in the LDH Cytotoxicity Reagent) LDH converts lactate to pyruvate and generates nicotinamide adenine dinucleotide (NADH). The WST-1 molecule, also present in the LDH Cytotoxicity Reagent, is converted from WST-1 to the orange formazan form. An increase in cell cytotoxicity is accompanied by increased LDH release and increased colorimetric signal. The assay principles are basic and can be applied to most eukaryotic cell lines, including adherent and non-adherent cells and certain tissues, depending on LDH expression levels. The LDH Cytotoxicity Reagent can be used to detect cytotoxicity in mammalian cells.



Related Products

1. CBA-232: Quantitative Cellular Senescence Assay (SA β-Gal)
2. CBA-240: Cell Viability and Cytotoxicity Assay
3. CBA-251: CytoSelect™ BrdU Cell Proliferation ELISA Kit
4. CBA-252: CytoSelect™ MTT Cell Proliferation Assay
5. CBA-253: CytoSelect™ WST-1 Cell Proliferation Assay Reagent

Kit Components

1. LDH Cytotoxicity Assay Reagent (Part No. 124101): One 10 mL bottle of reagent
2. Triton X-100 Solution (Part No. 124102): One 10 mL bottle of 10% Triton X-100

Materials Not Supplied

1. Cells for measuring cytotoxicity
2. Cytotoxicity mediating compound to be tested
3. Cell culture medium
4. 96-well clear cell culture plates
5. Microtiter plate reader capable measuring absorbance at 450 nm

Storage

The LDH Cytotoxicity Assay Reagent is a clear, slightly red, ready-to-use solution. Aliquot as needed to avoid repeated freeze-thaw cycles and store at -20°C protected from light. If precipitates or turbidity are observed upon thawing, warm the solution to 37°C for 5-10 minutes and agitate to dissolve the precipitates.

Store the Triton X-100 Solution at Room Temperature.

Assay Protocol

Note: Both a negative and a positive control should be run alongside experimental samples. Each experimental sample and control should be assayed in duplicate.

1. Prepare a cell suspension containing 0.1-1.0 x 10^6 cells/mL in medium.
2. Add the cell suspension to a 96-well cell culture plate for each experimental sample, negative and positive control well according to Table 1 below. Include the compound to be tested in experimental wells only.

	Experimental Sample	**Negative Control**	**Positive Control**
Cell Suspension	150 µL	150 µL	150 µL
Cytotoxicity-mediating compound to be tested	+	-	-

Table 1. Preparation of Experimental and Control Wells.

3. Culture the cells for 24-96 hours at 37°C and 5% CO_2 in a humidified incubator.
4. Add sterile water or the provided Triton X-100 Solution to each well according to Table 2 below.

	Experimental Sample	Negative Control	Positive Control
Sterile Water	15 µL	15 µL	-
Triton X-100 Solution	-	-	15 µL

Table 2. Lysis of Positive Control Cells.

5. Incubate 5-10 minutes at room temperature.
6. Transfer 90 µL of medium from each well to a clean 96-well plate suitable for a plate reader.
7. Add 10 µL of LDH Cytotoxicity Assay Reagent.
8. Incubate plate at 37°C and 5% CO_2 for 0.5-4 hours.
9. Read OD using 450 nm as the primary wave length.

Example of Results

The following figure demonstrates typical results with the LDH Cytotoxicity Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

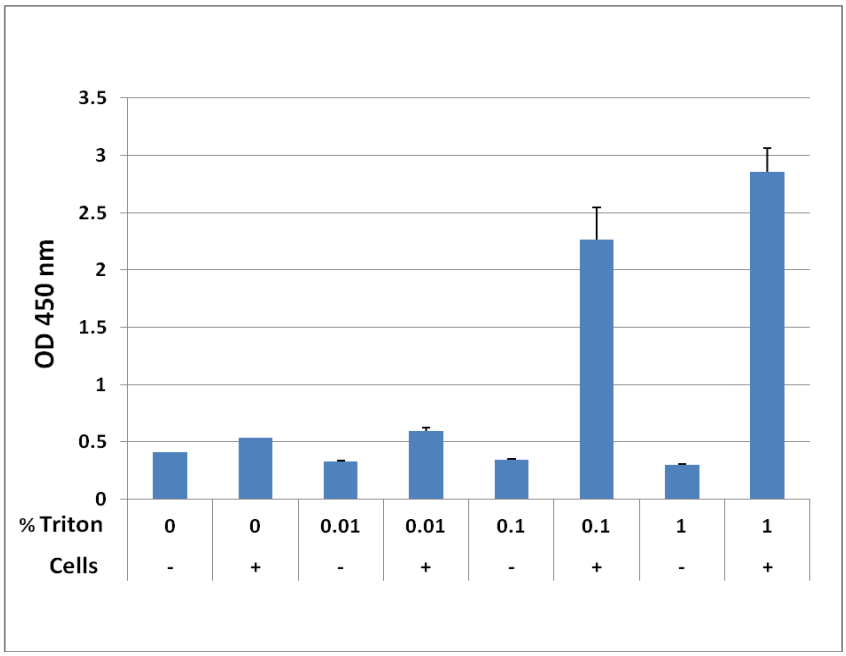


Figure 1. LDH Release from Human HEK 293 Cells. HEK 293 cells were seeded at 20000 cells per well and allowed to grow for 24 hours. After adding various concentrations of Triton X-100, LDH Cytotoxicity Assay Reagent was added. Cells were incubated for 30 minutes at 37ºC and 5% CO_2, and then read on a colorimetric plate reader.

Calculation of Results

Positive control wells represent maximal LDH release, while negative control wells represent background LDH release. The OD for negative controls is subtracted from both experimental and positive control OD values, and results are reported as a relative cytotoxicity percentage:

$$\frac{OD_{\text{experimental sample}} - OD_{\text{negative control}}}{OD_{\text{positive control}} - OD_{\text{negative control}}} \times 100 = \% \text{ Relative Cytotoxicity}$$

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Recent Product Citations

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