

LDH Cytotoxicity Assay

Cat. No: XV0020CA-F, XV0020CA, XV0022CA

Introduction

Lactate dehydrogenase is a ubiquitous enzyme present in cytosol of a wide variety of organisms. Since LDH is a fairly stable enzyme, it has been widely used to evaluate the presence of damage and toxicity of tissue and cells. Quantification of LDH has a broad range of applications. The measurement of this enzyme can be useful for estimating biomass, counting cells, and determining the health of cells and tissues. LDH is widely used for cytotoxicity testing of cultured cells as well as viability testing.

This assay kit uses the formazan dye INT as a colorimetric indicator of enzyme activity. LDH strips an electron from lactate which is transferred to NAD to yield NADH. The NADH subsequently reduces the INT which causes a color change from yellow to red. This colorimetric reaction can be measured at 490 nm.

Applications & Features

- Rapid determination of cytotoxicity based on LDH released into cell culture medium.
- Evaluation of toxic compounds, toxins, deterfents, environmental pollutants and physical treatment on cell lysis.
- Simple one-step procedure.
- Can be used for high-throughput screening.
- Colorimetric & non-radioactive.

Kit Contents

Components	400 Assays	2,000 Assays
LDH Assay Buffer	20 ml	5 x 20 ml
Lysis Solution	4 ml	20 ml
Stop Solution	20 ml	2 x 50 ml

Storage & Shelf Life

The kit is shipped on cool packs at +4°C, please see below for storage conditions.

- LDH Assay Buffer – store at -20°C, protected from light. Avoid freeze/thaw cycles.
- Lysis Solution & Stop Solution – store at +4°C.

Protocol

1. Cells are grown in a 96-well plate at a density of $1 \times 10^4 - 5 \times 10^4$ cells/well in 100 μ l growth medium.

Note: Assays can be performed on either adherent cells or cells in suspension.

2. On the same plate, prepare the following controls in triplicates.
 - **Untreated Cells Control:** Contain untreated cells to serve as a vehicle control. Add the same solvent used to deliver the test compounds.
 - **Lysis Control (Maximum LDH Activity):** Seed the same number of cells in these wells as in step 1.

Note: Different cell types contain different amounts of LDH. Therefore, the optimal cell density used should be determined empirically by performing a "total lysis" using different number of cells. The resulting LDH absorbance reading can be plotted against the cell number. The optimal cell number should be the one that falls within the linear range of the absorbance curve.

3. Add test compounds and vehicle controls to the appropriate wells.
4. Culture the cells for the required amount of time in a humidified 37°C incubator equilibrated with 5% CO₂.
5. To the lysis control wells (maximum LDH activity) add 10 μ l of 10X Lysis Solution and mix by gentle tapping.
6. Return the plate to a humidified 37°C, 5% CO₂ incubator for 45 minutes.
7. **[Optional]** Centrifuge the 96-well at 600 x g for 10 minutes.
8. Collect 50 μ l of culture supernatant from each well and transfer it to a new 96-well flat-bottom plate. Be careful not to transfer any cell materials.
9. Equilibrate LDH Assay Buffer to room temperature.
10. Add 50 μ l LDH Assay Buffer per well. Mix by gentle shaking for 30 seconds and incubate at room temperature for 10-30 minutes protected from light.
11. Add 50 μ l of Stop Solution to each sample well and mix.

Note: Perform measurement of the absorbance within one hour after addition of the Stop Solution. If the reaction rate is slow (i.e. slow color conversion), absorbance can be measured without adding the Stop Solution.

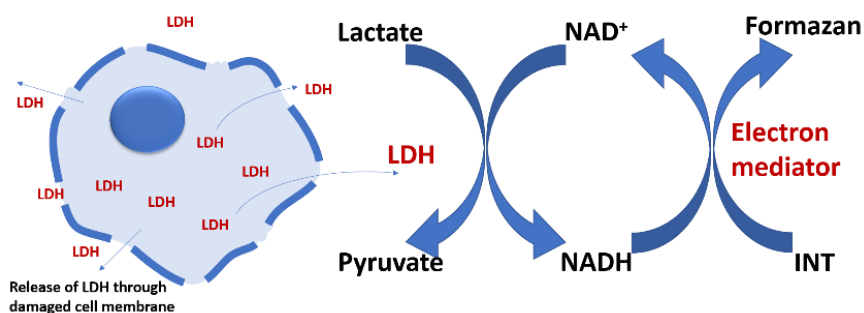
12. Measure the absorbance (OD) of all controls and experimental samples with a plate reader equipped with 490 nm filter. The reference wavelength should be 650 nm.

Note: Air bubbles present in the wells affect the absorbance readings. Break any bubbles present in the wells with a syringe needle and/or centrifugation before reading.

Data Analysis

Lysis Control wells represent maximal LDH release, while Untreated Cells control wells represent background LDH release. The OD for Untreated Cells control is subtracted from both experimental and Lysis Control OD values, and the results are reported as a relative cytotoxicity percentage:

$$\% \text{ Relative Cytotoxicity} = 100 \times \frac{\text{OD experimental sample} - \text{OD Untreated Cells Control}}{\text{OD Lysis control} - \text{OD Untreated Cells Control}}$$



PRODUCT USE LIMITATION This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.