

# LDH Cytotoxicity Assay Kit

## (Colorimetric Readout)

### Ordering info

TBK0521, 400 assays

TBK0522, 1.000 assays

### Description

LDH Cytotoxicity Assay Kit is a robust kit to measure cell death or cytotoxicity in cell culture experiments through lactate dehydrogenase (LDH) activity. LDH is a cytosolic enzyme that is rapidly released into the cell medium after damage of cell membrane. The amount of LDH released is proportional to the number of cells undergoing necrosis, apoptosis, or other forms of cell death, as well as from damaged or stressed cells.

LDH activity determination is based on the reduction of the tetrazolium salt, iodonitrotetrazolium chloride (INT). INT is reduced by LDH in the supernatant to form a colored formazan product that can be quantified spectrophotometrically at 490 nm.

### Features

- **Non-radioactive assay**, is an alternative to  $^{51}\text{Cr}$ -assay
- **Accurate, simple and reproducible** assay
- Ideal for **high throughput screening**
- **Measurement can be performed directly in the tissue culture medium** without solubilization process
- **Highly Sensitive**, with a limit of detection of 1-10 ng/mL of LDH
- Suitable assay for both **adherent and non-adherent cells**

### Kit Components

Components	TBK0521	TBK0522
LDH Lysis Solution <sup>a</sup>	5 mL	12 mL
LDH Reaction Buffer <sup>b</sup>	4 x 5 mL	10 x 5 mL
LDH Dye Solution <sup>b</sup>	320 $\mu\text{L}$	770 $\mu\text{L}$
LDH Stop Solution <sup>a</sup>	25 mL	55 mL

**Order Info Kit Components:** LDH Lysis Solution (TBB0565) | LDH Reaction Buffer (TBB0566) | LDH Dye Solution (TBR0266) | LDH Stop Solution (TBB0567).

### Storage

<sup>a</sup> Store at 4 °C.

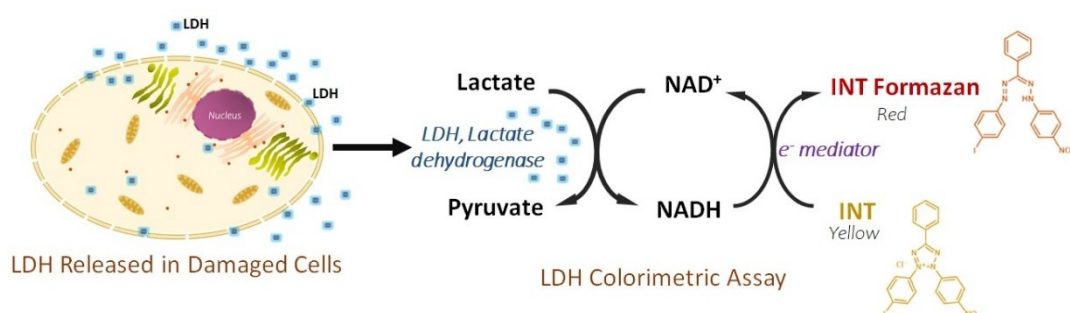
<sup>b</sup> Store at -20°C. Protect Solution from light.

### Quality Control

The kit is tested in a functional assay with LDH dilutions.

### Applications

- For assessing -induced cytotoxicity of drugs/ pollutants/ test compounds
- To assess the cytotoxicity of immune cells (NK, T lymphocytes, etc) or viruses against target cells
- To evaluate the invasiveness of cancer cells
- Antibody-dependent cell-mediated cytotoxicity
- Cell death, cell viability or cell proliferation measure



## PROTOCOL

### I. REAGENT PREPARATION

#### LDH Working Solution (100 assays)

1. Equilibrate LDH Reaction Buffer and LDH Dye Solution to room temperature.
2. Prepare fresh, immediately before its use, adding **75  $\mu$ L Dye Solution** to **5 mL LDH Reaction Buffer**.
3. Mix well by inversion and protect from light.

### II. DETERMINATION OF OPTIMAL CELL NUMBER

As the level of LDH protein is variable in different cell lines, it is convenient to perform a cell titration experiment to determine the optimal cell density for the cell line you are going to use.  $1-5 \times 10^4$  cells/well is optimal for many cell lines.

1. In a 96-well plate, distribute two sets of serial cell dilutions (0-20,000 cells/well, 100  $\mu$ L/well). Each dilution must be in triplicate.
2. **Lysis Control (Maximum LDH Release):** Add **10  $\mu$ L LDH Lysis Solution** to one set of serial cell dilutions and mix gently by tapping.
3. **Spontaneous LDH release.** Add **10  $\mu$ L of medium** to the other set of serial dilutions and mix gently.
4. Incubate at 37°C, 5% CO<sub>2</sub>, for 45 minutes.
5. [**Optional, but recommended**] Centrifuge at 600 g, 10 minutes.
6. Proceed with LDH Assay.

### III. CYTOTOXICITY ASSAY

1. In a 96-well plate, grow the cells in 100  $\mu$ L medium at the density determined experimentally. Seed enough wells to include the assay controls and samples in triplicate.

*Both human and animal sera contain LDH, which may increase background absorbance in the assay. It is recommended to conduct the assay in the presence of low serum (e.g., 1%).*

2. Incubate the cells at 37°C, 5% CO<sub>2</sub> for 24 hours.

#### **Samples**

3. Add **10  $\mu$ L of compounds** to be tested to the corresponding well.

#### **Controls**

4. Set up the following controls:
  - **Lysis Control:** It is a measure of the maximum releasable LDH in the cells.
  - **Background Control:** It is a measure of LDH activity of untreated cells.
  - **Positive Control:** It is a measure of maximum LDH released by using a compound that mimics the physiological mechanism of cell death being studied. Some commonly specific compounds used as positive controls are staurosporine (1-10  $\mu$ M), hydrogen peroxide (0,1-1 mM), doxorubicin (1-10  $\mu$ M).

	Lysis Control	Background Control	Positive Control
Cells/ Well	100 µL	100 µL	100 µL
LDH Lysis Solution	10 µL	-	-
Compound Vehicle	-	10 µL	-
Specific Compound	-	-	10 µL

- Incubate the plate at 37 °C in a humidified incubator with 5% CO<sub>2</sub> for the length of time required by your experiment to induce cytotoxicity (6 - 48 h).
- Proceed with LDH Assay.

#### IV. LDH ASSAY

- Equilibrate 2x Reaction Buffer at room temperature.
- Transfer **50 µL of culture supernatant** to a fresh 96-well plate flat bottom.
- Add **50 µL LDH Working Solution** per well and mix well.
- Incubate at room temperature for 10-30 minutes in the dark. Protect the plates from light.
- Add **50 µL LDH Stop Solution** to each well and mix.
- Read at 490 nm in a plate reader  
*Avoid the presence of burbles in the wells before reading.*

#### V. LDH ACTIVITY DETERMINATION

Calculate the percent of cytotoxicity by the following equation:

$$\text{Cytotoxicity (\%)} = \frac{\text{Absorbance of Samples} - \text{Background Control}}{\text{Absorbance of Lysis Control} - \text{Background Control}} \times 100\%$$