



Assay kits

LDH Cytotoxicity Assay kit

Determination of cellular toxicity in vitro

Protocol



Notes :

LDH Cytotoxicity Assay Kit | Specifications

Package content	LDH0500 : 12.5mL of 2X INT Buffer (A) 12.5mL of 2X Assay Buffer (B) 100µL of 1000X Buffer (C) 25mL Stop Solution 1mL Lysis Buffer (10X) 10µL positive control Number of assays (96-well plate) : 500
Shipping conditions	The kit is shipped at RT.
Storage conditions	Upon reception, store the Lysis Buffer and Stop solution at 4°C and other components of the LDH cytotoxicity kit at -20°C. Protect from light and avoid freeze-thaw cycles
Stability	1 year at -20°C.

Technology

Description

The **LDH cytotoxicity Kit** is a colorimetric assay for the determination of cytotoxicity *in vitro*. This assay kit measures the release of lactate dehydrogenase (LDH) from damaged and lysed cells in surrounding culture medium. LDH is a stable cytosolic enzyme that allows the conversion of iodinitrotetrazolium (INT), a tetrazolium salt into formazan product in presence of an electron mediator. The amount of formazan produced is directly proportional to the release of LDH and thus, to lysed cells. As opposed to other cell assay kits, no solubilisation process is required since this formazan does not require any solvation; measurement can be performed directly in the tissue culture medium.

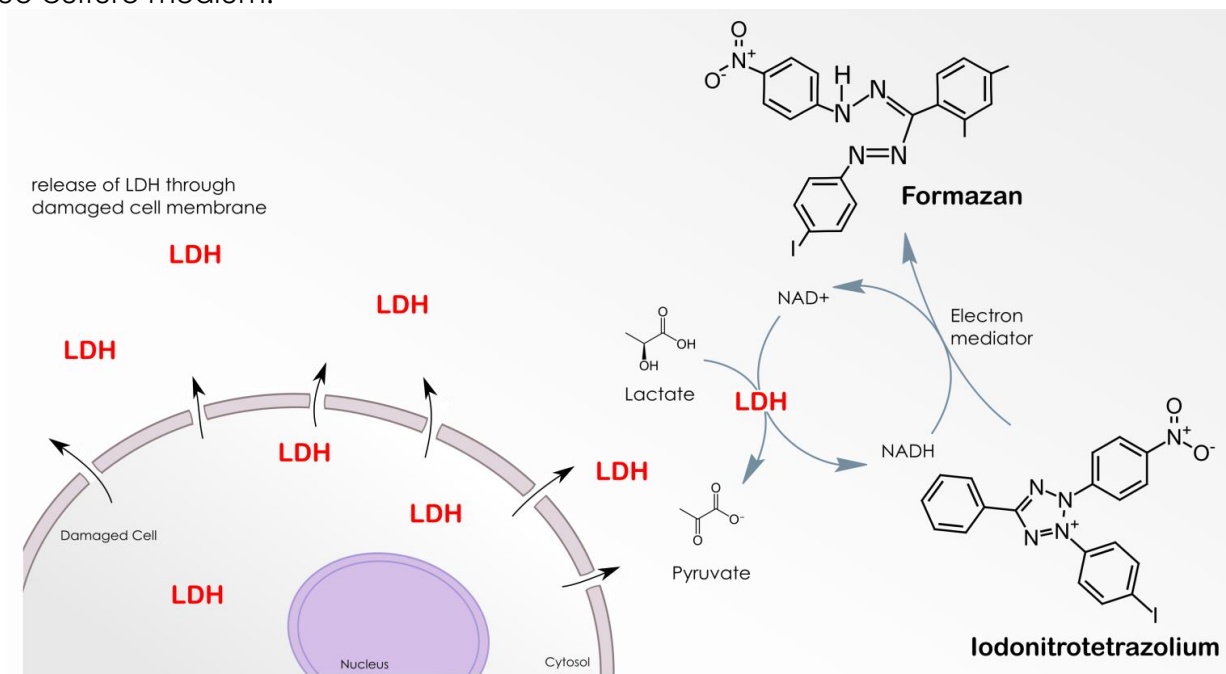


Figure 1: Membrane-damaged cell releases LDH that catalyses the formation of red formazan from INT. Formazan that is proportional to the amount of LDH produced is measured using the LDH cytotoxicity kit.

The **LDH cytotoxicity Kit** is more sensitive at neutral pH than other tetrazolium salts such as MTT, XTT, MTS or WST-1. The amount of formazan produced is directly proportional to the number of living cells. As opposed to WST-8 kit that uses the same read-out and offers the possibility to monitor cell proliferation, LDH cytotoxicity kit is more dedicated to measuring cell toxicity, late apoptosis or necrosis.

Colorimetric measurement at 490 nm allows the quantification of toxicity. The **LDH cytotoxicity Kit** does not need cell fixation, cell lysis or washing steps rendering this ready-to-use kit fast, accurate, sensitive and adapted to high throughput screening.

Applications and Protocol

1. General Considerations

- Serum present in complete culture medium may affect LDH activity causing background signal in the assay.
- Avoid repeated freeze-thaw cycles that could decrease the enzyme efficiency resulting in an increase of the background signal.
- This kit is compatible with phenol red containing medium.
- Incubation time varies with the type and number of cells.
- Allow reagent to reach room temperature before starting.
- Avoid direct exposure to- and protect from light.

2. Reagent Preparation

1. Working solution:

Prepare **5mL** of Working Solution, sufficient for one 96-well plate:

- Combine **2.5mL** of 2X INT Buffer (A) with **2.5mL** of 2X Assay Buffer (B)
- Add **5µL** Buffer (C) for a 1X final
- Mix gently and protect from light until use

NOTE: Unused working solution can be stored at -20°C , protected from light for a couple of weeks. Avoid freeze/thaw cycles.

2. LDH Positive Control

Prepare 1X LDH positive control: Dilute **10µL** of LDH positive control in **5mL** of PBS 1%BSA.

Aliquote and store at -20°C .

3. General Protocol

1. Cell plate preparation:

- a. Seed cells in a culture plate under standard culture conditions
- b. Fill 3 wells with culture medium only for culture medium background control
- c. Carry out experiment by adding chemical compounds or biological agents to cells
- d. Leave 3 wells unstimulated for maximum LDH release control measurement.

NOTE: we recommend performing measurement in triplicates.

2. Measuring cell cytotoxicity:

- a. Collect **50µL** of each sample medium supernatant into a 96-well flat bottom plate
- b. Add **50µL** of Working Solution to each well
- c. Incubate **20min** to **1h** at room temperature in the dark (incubation time varies depending on the metabolic activity of the cells)
- d. Stop the reaction by addition of **50µL** of Stop solution

*NOTE: proceed to reading within the **2h** windows after addition of stop solution*

- e. Quantify the release of LDH by measuring absorbance at **490nm**
- f. Subtract background absorbance measured at **680nm** from all values.

3. Controls Measurement (optional but recommended):

- a. Culture medium background control

Measure LDH activity in the 3 wells filled only with culture medium. This would allow correcting basal LDH activity that may be present in serum of complete culture medium.

- b. Positive control measurement

Add **50µL** of 1X LDH positive control to 3 empty wells and measure LDH activity.

- c. Maximum LDH release control (100% release of LDH)

Add **1X final** Lysis Buffer to the 3 wells containing unstimulated cells let for maximum LDH release control measurement

Incubate 30-45 minutes under standard culture conditions to insure a complete cell lysis

Transfer **50µL** of supernatant and measure LDH activity.

Optionally, you can perform a centrifugation step to lower the background signal due to cell debris.

This control can be used to calculate the % of cytotoxicity:

$$\% \text{ Cytotoxicity} = \frac{\text{Cell sample-culture medium background}}{\text{Maximum LDH release}} \times 100$$

Optionally for suspension cells, a centrifugation procedure to pellet cells (5min x 1200rpm) would allow working on supernatant to lower background signal.

4. Performance characteristics

1. Sensitivity of the LDH assay kit.

This kit is able to detect as low as 0.01 u/mL of purified recombinant mammalian LDH produced in E.Coli.

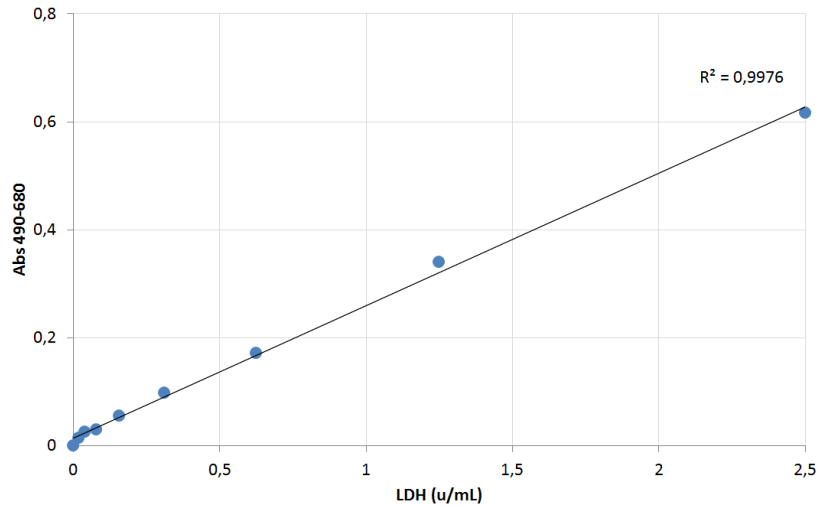


Figure 2: Dosage of the activity of recombinant mammalian lactate dehydrogenase (LDH). Standard curve of LDH from 0 to 2.5u/mL was realized by serial two-fold dilutions in 50 μ L H₂O in triplicate. 20min after addition of 50 μ L working solution, the absorbance was read at 490nm and 680nm to determine LDH activity.

2. Maximum LDH release depending on cell number.

In the following experiment, LDH lysis buffer was used to determine the linear range of the LDH cytotoxicity by measuring the spontaneous LDH release in HeLa cells. Culture medium was removed and replaced with 1X final lysis buffer to avoid background signal.

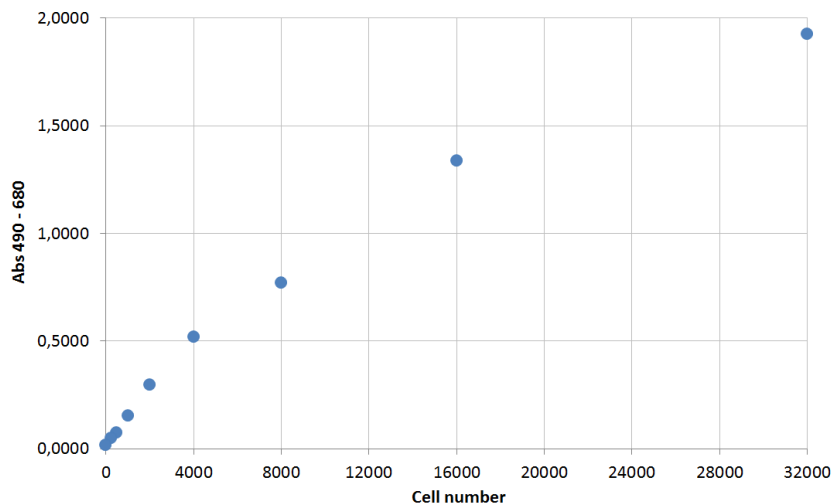


Figure 3: LDH activity determined in HeLa cells: 24H after seeding, medium was removed and cells were lysed using the LDH lysis buffer (1X) and LDH activity was measured according to the protocol after 20 min incubation.

3. Toxicity measurement in stem cell line KG1a after H2O2 stimulation.

This kit is compatible with both cell suspension and adherent cells. Cell toxicity was measured after stimulation of KG1a cell line cultivated in suspension with ranging doses of H2O2 to induce cell death.

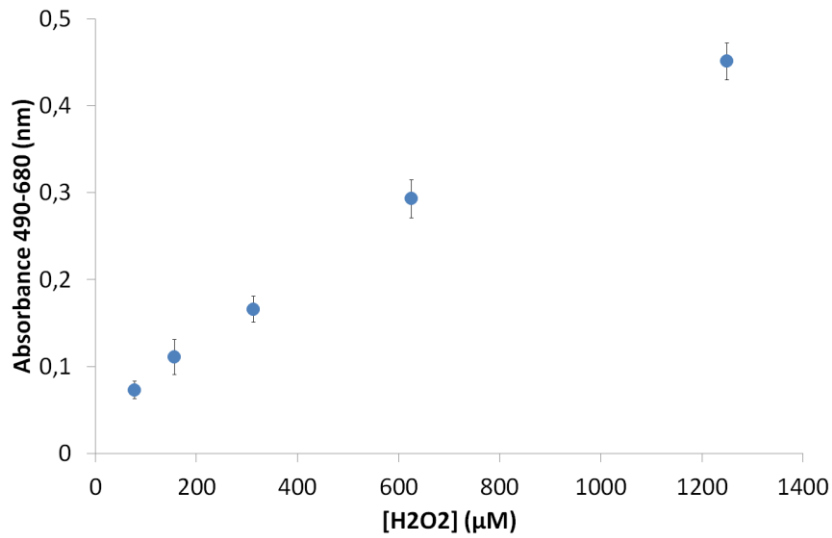


Figure 4: Cell toxicity confirmed by measuring LDH activity in KG1a cells after H₂O₂ stimulation (40min incubation time).

Additional products:

- **ROS Assay Kit** for the quantification of Reactive Oxygen Species
- **OZBlue Cell Viability Kit** for the quantification of metabolic activity in living cells
- **MTT Cell Proliferation Kit** for the quantification of cells growth
- **Cellular Senescence Kit** for the determination of cellular senescence

Purchaser Notification

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