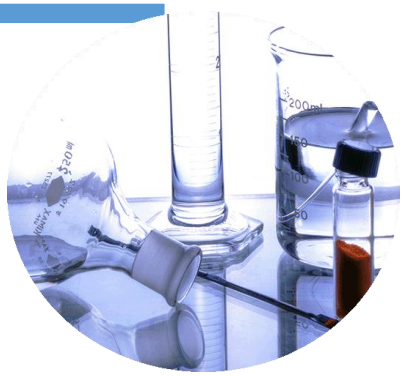
LDH Cytotoxicity Assay Kit

INSTRUCTION MANUAL

Assays kits



LDH Cytotoxicity Assay Kit

The **LDH Cytotoxicity Assay Kit** is a colorimetric assay kit for the determination of cellular toxicity *in vitro*.

	Content	Catalog Number	Number of assays (96-well plate)
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	**LDH0500**	500

For any technical questions, contact us at tech@ozbiosciences.com

OZBIOSCIENCES
The art of delivery systems

1. Technology

1.1. 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 iodonitrotetrazolium (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.

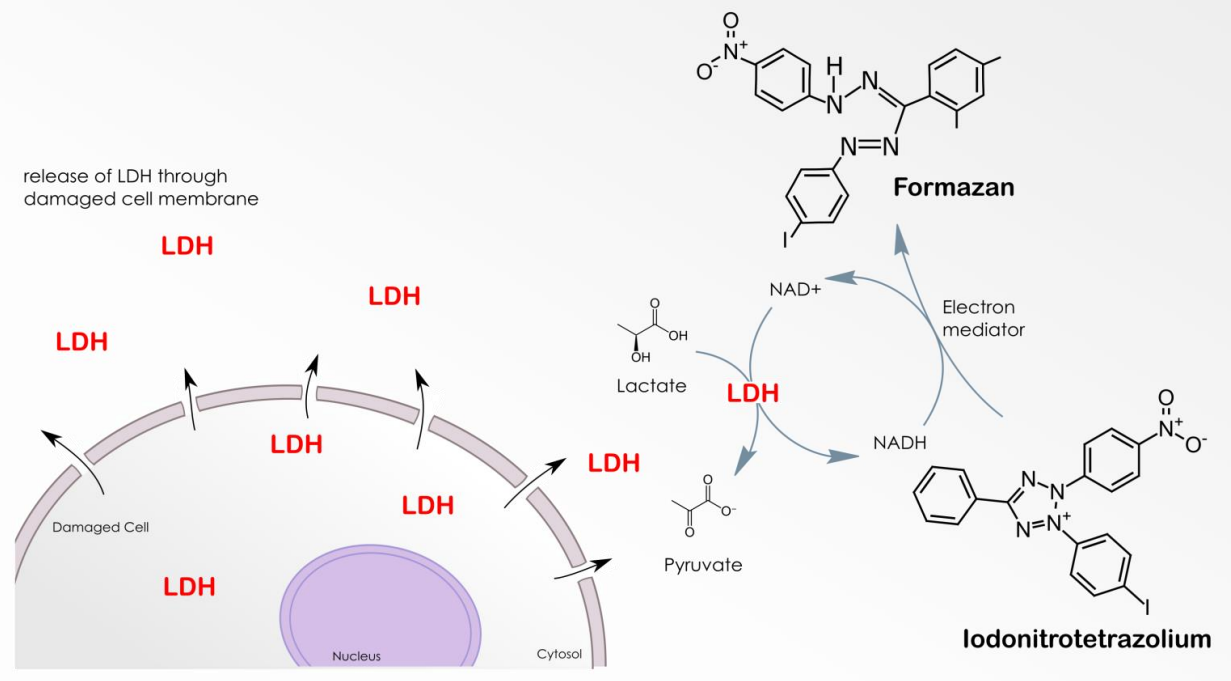


Figure 1: Membrane-damaged cell releases LDH that catalyses the formation of red formazan from INT. Formazan that is proportional to the amount of LDH produced is measured using the LDH cytotoxicity kit.

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.

1.2. Storage and shipping condition

Storage: 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.

Shipping condition: The kit is shipped at RT.

2. Applications and Protocols

2.1. 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.

2.2. 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.

2.2. General protocol

A. Cell plate preparation:

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

NOTE: we recommend performing measurement in triplicates.

B. Measuring cell cytotoxicity:

1. Collect **50µL** of each sample medium supernatant into a 96-well flat bottom plate

2. Add **50µL** of Working Solution to each well
3. Incubate **20min** to **1h** at room temperature in the dark (incubation time varies depending on the metabolic activity of the cells)
4. Stop the reaction by addition of **50µL** of Stop solution

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

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

C. Controls Measurement (optional but recommended):

1. 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.

2. Positive control measurement

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

3. 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.

2.4. Performance characteristics

Sensitivity of the LDH assay kit.

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

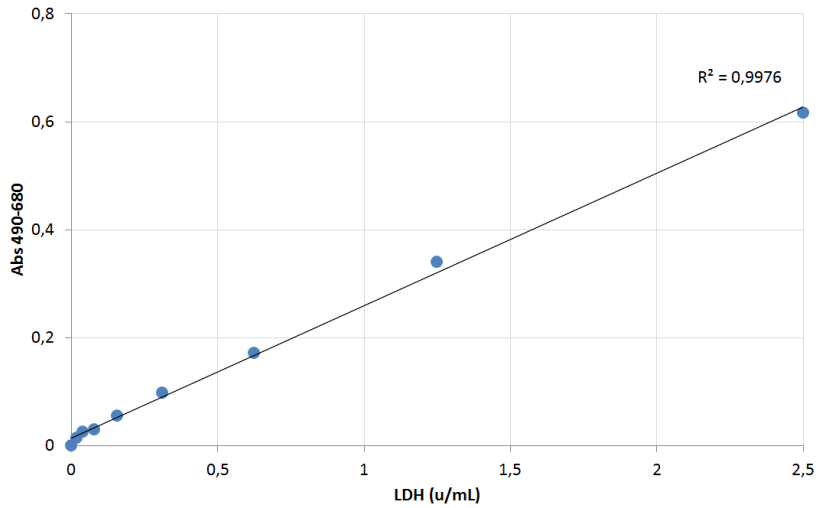


Figure 2: Dosage of the activity of recombinant mammalian lactate dehydrogenase (LDH). Standard curve of LDH from 0 to 2.5u/mL was realized by serial two-fold dilutions in 50µL H2O in triplicate. 20min after addition of 50µL working solution, the absorbance was read at 490nm and 680nm to determine LDH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

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.

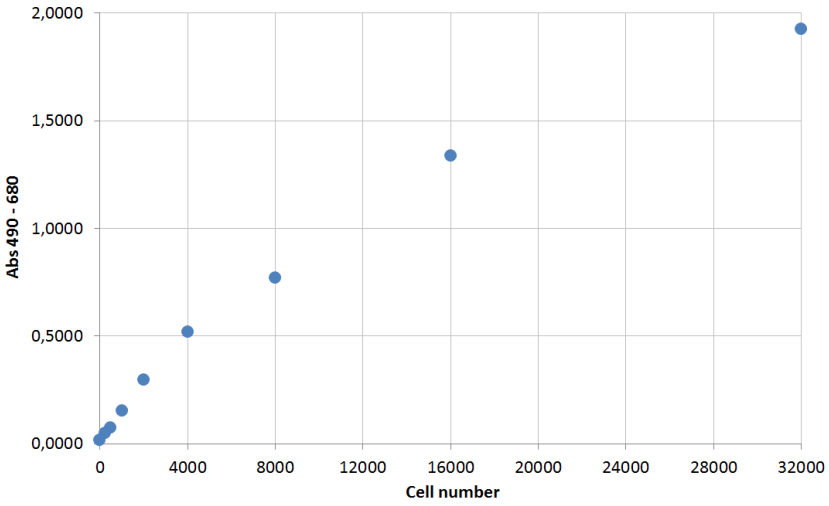


Figure 3: LDH activity determined in HeLa cells: 24H after seeding, medium was removed and cells were lysed using the LDH lysis buffer (1X) and LDH activity was measured according to the protocol after 20 min incubation.

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.

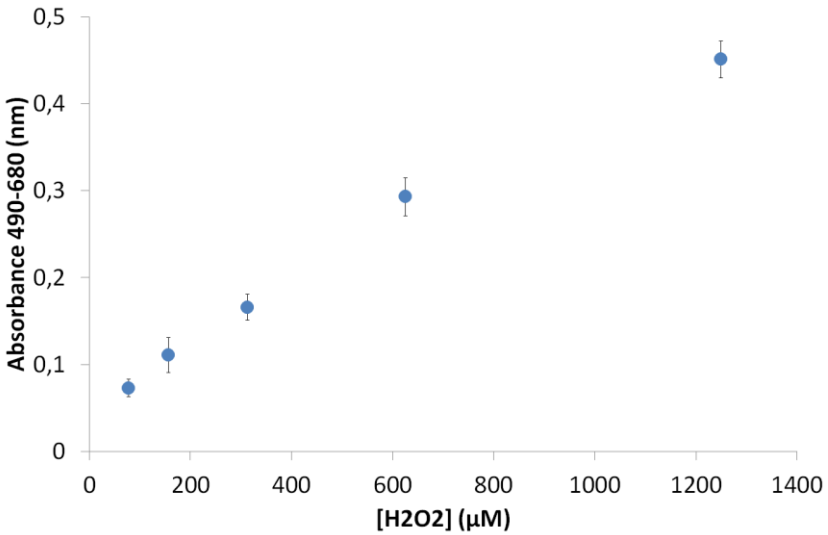


Figure 4: Cell toxicity confirmed by measuring LDH activity in KG1a cells after H2O2 stimulation (40min incubation time).

Purchaser Notification

Limited License

The purchase price paid for **LDH cytotoxicity Kit** by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the section 1, Kit Contents). These reagents are intended **for internal research only** by the buyer. Such use is limited to the use in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

Purchasers may terminate this License at any time by returning all **LDH cytotoxicity Kit** material and documentation to OZ Biosciences, or by destroying all **LDH cytotoxicity Kit** components. Purchasers are advised to contact OZ Biosciences with the notification that a **LDH cytotoxicity Kit** is being returned in order to obtain a refund and/or to expressly terminate a research only license granted through the purchase of the kit(s).

This document covers in full the terms of the **LDH cytotoxicity Kit** research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The **LDH cytotoxicity Kit** and all its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Business Development
OZ Biosciences SAS
Parc Scientifique et Technologique de Luminy
Bâtiment Grand Luminy technopole
Case 922 zone entreprises
13288 Marseille Cedex 9, France
Ph: +33 (0)4.86.94.85.16
Fax: +33 (0)4.86.94.85.15
E-mail: contact@ozbiosciences.com

Related Products

PLASMIDS – REPORTER GENES
pVectOZ-LacZ
pVectOZ-SEAP
pVectOZ-GFP
pVectOZ-Luciferase
ASSAY KITS
Senescence / Viability / Stress
ROS Detection Assay Kit
OZBlue Cell Viability Kit
MTT Cell Proliferation Kit
Senescence Kit for Stem Cells
Protein Dosage
FluoProdige Protein Quantification Assay Kit
Bradford PaK
BCA PaK
Reporter Gene
SEAP Assay Kit
Luciferase Assay Kit
β-Galactosidase assay kits (CPRG/ONPG/X-Gal Staining Kit)

CONTACTS

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
FRANCE

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117
USA

Ph : + 1-858-246-7840
Fax : + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

http://www.ozbiosciences.com

Follow us!