LDH-Cell Cytotoxicity Assay Kit

(100 assays)

Zellbio GmbH (Germany)

CAT No. ZX-44118-100

www.zellx.de

Application:

Detection of cellular cytotoxicity

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

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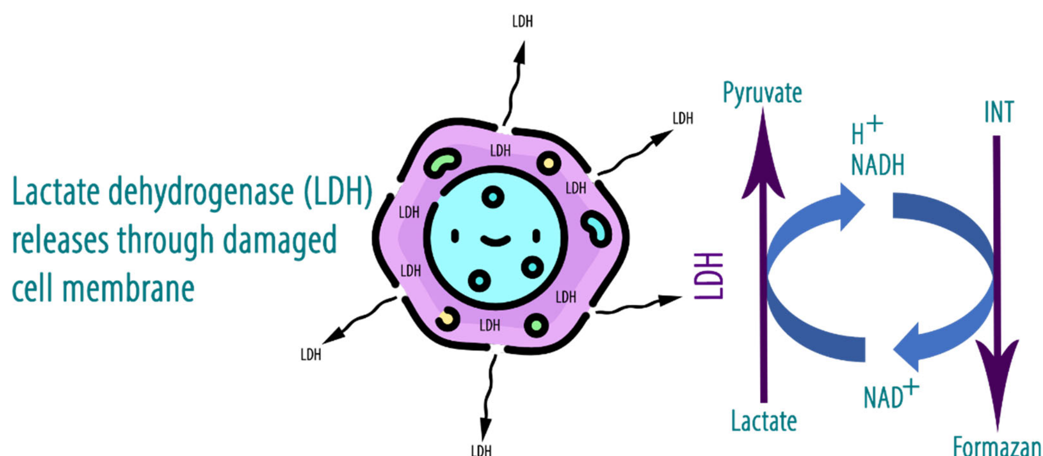
Background

Lactate dehydrogenase (LDH) is a soluble enzyme which is present in the cytosol of a wide variety of organisms. When cells are cultured *in vitro*, LDH can be released into the culture medium upon the loss of membrane integrity due to cell- or compound-induced cytotoxicity. Since LDH is a stable enzyme, its presence in culture media indicates the loss of cell membrane integrity in response to damage and toxicity. LDH measurement is used to analyze cellular viability and viable cell count, estimate biomass, evaluate the cytotoxicity in cultured cells and determine the health of cells and tissues.

ZellX® LDH Cell Cytotoxicity Colorimetric Assay Kit uses the formazan dye iodonitrotetrazolium (INT) as a colorimetric indicator of enzyme activity. LDH catalyzes the oxidation of lactate to pyruvate via reduction of NAD+ to NADH and H+. The NADH subsequently reduces the INT (yellow) to the highly colored formazan (red) which can be measured at 490 nm.

Figure: LDH is released ….



Intended use

Determination of cellular cytotoxicity and cell lysis in response to drugs, toxic compounds, toxins, detergents, environmental pollutants, and physical treatment.

Materials supplied in the Kit

Component	***Quantity***
LDH Assay Buffer	5 mL
Dye Solution	75 µL
Cell Lysis Solution (10X)	1 mL
Stop Solution	5 mL

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Storage instruction

All reagents, except for the **LDH Assay Buffer** and **Dye Solution**, should be stored at 4° C until the expiration date of the kit. **LDH Assay Buffer** and **Dye Solution** must be stored at -20° C, Avoid freezing/thawing cycles.

Materials required but not supplied

Precision pipettes and disposable filter pipette tips

Sterile clear 96 well plate

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Reagent preparation

i. **LDH Working Solution:** Add 75 µL of the Dye Solution to 5 mL of LDH Assay Buffer (this amount is sufficient for one 96-well plate or 100 assays). Mix well by inverting gently and protect from light. LDH Working Solution must be used immediately after preparation.

Sample preparation

i. At an appropriate time before the experiment, cultivate cells ($1*10^4$-$5*10^5$ cells/well) in a 96-well plate containing 100 µL/well of cell culture medium. The appropriate time of incubation may vary depending on the cell types and the desired chemical or physical treatments and must be evaluated by the end user.

ii. Add test compounds and solvent in triplicate wells and incubate the cells in a CO_2 incubator at 37°C for an appropriate time.

iii. Each plate must include at least three wells as **Untreated Cells Control** which should be treated with the same solvent but without the potential cytotoxic component; and three wells as **Lysis Control** whereby the same number of cells must be cultivated to detect the maximum LDH activity when they are lysed.

- We strongly recommend to determine the optimal cell number and incubation time for your specific cells before performing a large number of LDH assays.

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Assay Procedure

1. Forty-five minutes before starting the experiment, add 10 µL of 10X Cell Lysis Solution to Lysis Control wells, tap the plate and mix gently.
2. Incubate at 37°C in a CO_2 incubator for 45 minutes.
3. Centrifuge the 96-well at 600 g for 10 minutes.
4. Collect 50 µL of culture supernatant from each well and transfer to a new 96-well clear flat-bottom plate. Be careful not to transfer any cell debris.
5. Add 50 µL of the LDH Working Solution to each well. Mix gently for 30 seconds.
6. Incubate at room temperature protected from light for 10-30 minutes.
7. Add 50 µL of Stop Solution to each well and mix gently.
8. Read the absorbance signal at 490 nm (440-490 nm is acceptable). Read background absorbance at 650 nm.

Calculation

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 results are reported as a relative cytotoxicity percentage:

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

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