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DATA SHEET

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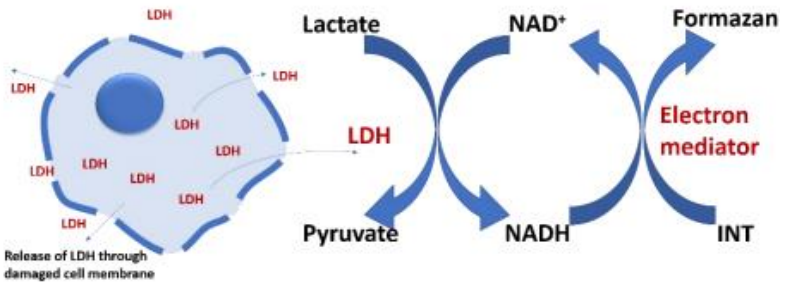
1. Identification

Product name **LDH Cytotoxicity Assay Kit (Colorimetric)**

Cat. No: **CA0020** 400 assays
CA0022 2000 assays

2. Description

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.



3. Composition

Item	Quantity (400 assays)	Quantity (2000 assays)
LDH assay Buffer	4 x 5 mL	4 x 25 mL
Dye Solution	4 x 75 µL	4 x 375 µL
Lysis Solution*	4 mL	20 mL
Stop Solution*	20 mL	100 mL

*Both products are storage at different temperature than the others, please see next section.

4. Storage Specifications

- Store LDH assay Buffer and Dye solution at -20˚C, protected from light.
- Store Lysis solution and Stop solution at +4˚C.

5. Applications

- For rapid determination of cytotoxicity based on lactate dehydrogenase released into cell culture medium.
- Evaluation of toxic compounds, toxins, detergents, environmental pollutants, and physical treatment on cell lysis.

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6. Product Feature

- Simple one-step procedure.
- Fast and convenient.
- Colorimetric measures LDH release by formation of colored product.
- Robust uses stable LDH enzyme activity as a cytotoxic marker.
- High-throughput screening.
- Non-radioactive.

7. Reagent Preparation

- REACTION MIXTURE: Equilibrate LDH assay buffer and Dye solution to room temperature.
- For 100 assays (96-well Microplates), mix 75 µl of dye Solution with 5 ml of LDH assay Buffer. Mix well by inverting gently and protect from light until use. The mixture solution should be prepared immediately before use.

8. Assay Procedure

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 µl growth medium.

Note: Assays can be performed on either adherent cells or cells in suspension. It is recommended to perform the assay in the presence of low serum concentration (e.g., 1%) or to replace serum by 1% bovine serum albumin (BSA). That is because serum contains various amounts of LDH, which may increase background absorbance in the assay.

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 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 µL 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 µL 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. Add **50 µL of Reaction Mixture** per well. Mix by gentle shaking for 30 seconds and incubate at room temperature for 10-30 minutes protected from light.

10. Add **50 µL 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 rate of the reaction rate is slow (i.e., slow color conversion), absorbance can be measured without adding the stop solution.

11. Measure the absorbance (OD) of all controls and experimental samples with a plate reader equipped with 450 nm (440 nm to 490 nm) filter. The reference wavelength should be 650 nm.

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

12. 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 results are reported as a relative cytotoxicity percentage:

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

9. Further Information

Product use Limitations

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

Disclaimer

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