BioChain®
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User's Manual and Instructions

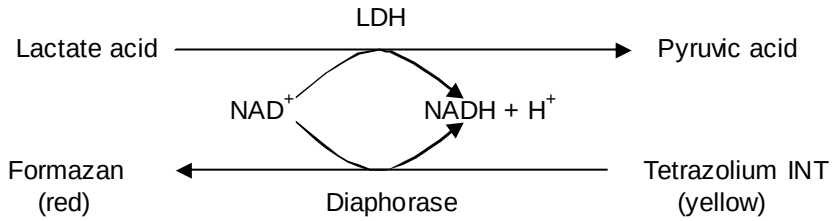
LDH Cytotoxicity Assay Kit

Catalog Number: K6330400

Introduction

Cell death is assayed by the quantification of plasma membrane damage. One of the standard methods is the measurement of cytoplasmic enzyme activity released from damaged cells. The amount of enzyme activity correlates to the proportion of the damaged cells. BioChain's LDH Cytotoxicity Assay kit is a colorimetric assay that quantitatively measures lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released into the cell culture supernatant upon damage of the cytoplasmic membrane. Released LDH in culture supernatants is measured with a 20-minute coupled enzymatic reaction: LDH oxidizes lactate to pyruvate which then reacts with tetrazolium salt INT to form formazan. The increase in the amount of formazan produced in culture supernatant directly correlates to the increase in the number of lysed cells. The formazan dye is water-soluble and can be measured by 490 nm absorbance using a standard ELISA plate reader. The LDH-cytotoxicity assay is fast, sensitive, convenient, and precise, and is applicable to a variety of cytotoxicity studies.

Figure 1: Principle of LDH Cytotoxicity Assay



Features

- **Safe** – No radioactive isotopes are required
- **Easy and Simple** - Add solutions to the cells, incubate and read the absorbance. The entire assay is performed in one microtiter plate with no washing and cell harvesting.
- **Fast** – Short reaction time, no centrifuge needed
- **Accurate** – Absorbance strongly correlates the number of lysed cells.
- **Sensitive** – More sensitive than alkaline and acid phosphatase-based assays.
- **Reliable** – Super-quality and highly reproducible data

Applications

- Measurement of cytotoxic effects of cytotoxic and cytostatic compounds such as anti-cancer drugs and many other toxic drugs.
- Can be used in fast, high-throughput drug screening.

Description

Components in this kit are prepared with pure chemicals according to our proprietary technology. BioChain's LDH Cytotoxicity Assay Kit enables to measure the cytotoxicity with colorimetric assay and based on based on the measurement of activity of lactate dehydrogenase (LDH) released from damaged cells. The procedures are simple, fast, sensitive and accurate. One kit is consisted of reagents enough for performing 400 assays.

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Quality Control
1 kit of this lot has been tested to go through the complete cytotoxicity assay. The 490 nm absorbance of cell supernatant using this lot of kit is comparable to those obtained with control (previous) lot.

Components
LDH Cytotoxicity Assay Kit: Reagents are sufficient for 400 assays.
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Item	Amount	Part No.
1. Catalyst Reagent (containing Diaphorase and NAD^+)	1 Vial	K6330400-1
2. Catalyst Reconstitution Buffer	6 ml	K6330400-2
3. Dye Solution (containing tetrozolium INT and lactate)	12 ml	K6330400-3
4. Lysis Solution (10x)	5 ml	K6330400-4
5. Stop Solution	15 ml	K6330400-5

Reagents and Equipments Required but not Supplied in this Kit:
1. Cell incubator
2. ELISA plate reader with 490 – 492 nm filter
3. Hemocytometer
4. Centrifuge with rotor which can fit micortiter plate

Storage and Stability
Store at -20 ºC. The kit is stable for one year when handled properly.

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Protocol

Reagent Preparation and Storage
Dissolve 1 vial of Catalyst reagent with 6 ml of Catalyst Reconstitution Buffer, aliquot the Catalyst solution and stored at -20 °C. Divide Dye Solution into working aliquots and stored at –20 °C, protected from light.

Assay medium: 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.

Assay Mixture: For 100 assays, mix 1.5 ml of Catalyst Solution with 3 ml of Dye Solution. The mixture solution should be prepared immediately before use.

1x Lysis Solution: Dilute 10x Lysis Solution 10-fold with assay medium. The maximum amount of releasable LDH enzyme activity can be determined by damaging cells with 1x Lysis Solution.

Assay Procedures

1. Collect cells and wash once with assay medium.
2. Preparing the following controls and test samples
   The following three controls have to be performed in each experimental setup in order to calculate percent cytotoxicity. (See table 1)
   Background control: Measure the LDH activity contained in the assay medium. The absorbance value obtained in this control has to be subtracted from all other values. Add 200 µl assay medium/well into triplicate wells.
   Low control: Measure the spontaneous LDH release, that is the LDH activity released from the untreated normal cells . Add 1 – 2 x 10^4 cells/well in total 200 µl assay medium into triplicate wells.
   High control: Measure the maximum releasable LDH activity in the cells, that is the maximum LDH release induced by the addition of 1x Lysis Solution. Add 1 – 2 x 10^4 cells/well in total 200 µl assay medium containing 1x Lysis Solution.
   Test Sample: Add 1 – 2 x 10^4 cells/well in total 200 µl assay medium containing test substance into triplicate cells.
   Amounts of released LDH differ depending on cell types. Therefore, the optimum cell concentration for a specific cell type should be determined in a preliminary experiment. In general, this cell concentration is at the point where the difference between the low and high control is at a maximum. With most cell lines, the optimal cell concentration is between 1 – 2 x 10^4 cells/well in 200 µl.

Contents	Background Control	Low Control	High Control	Test Sample
Cells in Assay Medium	---	100 µl	100 µl	100 µl
Assay Medium	200 µl	100 µl	---	---
Lysis Solution			100 µl	
Test Substance in Assay Medium	---	---	---	100 µl

3. Incubate the cells in a standard culture condition for the time applied in assaying the test substances.
4. Centrifuge the microtiter plate at 250x g for 10 min.
5. Remove 100 ul supernatant/well carefully (do not disturb the cell pellets) and transfer into corresponding cells of an optically clear 96-well flat bottom microtiter plate.
6. Add 45 ul of Assay Mixture to each well and incubate at room temperature for 30 min (protected from light).
7. (Optional) The reaction can be stopped by adding 50 µl of Stop Solution.

8. Measure the absorbance of the sample at the wavelength of 490 – 492 nm. If a reference wavelength is to be subtracted, a filter above 600 nm is recommended.

Calculation of Percentage Cytotoxicity

The percentage cytotoxicity is determined by calculating the average absorbance values of the triplicate subtracted with average background absorbance obtained from background control. The resulting values are substituted into the following equation:

$$\text{Cytotoxicity (\%)} = \frac{\text{exp. value - low control}}{\text{high control - low control}} \text{ X 100}$$

Related Products

WST-1 Cell Proliferation Assay Kit

References

1. Decker, T. & Lohmann-Matthes, M.L. J. Immunol Methods. 1988. 15: 61-69.
2. Korzeniewski, C. & Callewaert, D.M. J. Immunol Methods. 1983. 64: 313-320.
3. Nachlas, M.M. et al. Anal. Biochem.1960. 1:317-326.