Technical Manual

Lactate Dehydrogenase (LDH) Cytotoxicity Colorimetric Assay Kit

- Catalogue Code: MAES0196
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Lactate dehydrogenase (LDH) is a stable enzyme normally found in the cytosol of all cells but rapidly releases into the supernatant upon damage of plasma membrane. The quantitative of cytotoxicity can be analyzed by detecting the activity of LDH in the cell culture medium released from plasma membrane -ruptured of cells.

3. Intended Use

This kit can be used to measure lactate dehydrogenase (LDH) release in cytotoxicity.

4. Detection Principle

Lactate dehydrogenase catalyzes the reaction of lactic acid with NAD^+ to produce pyruvic acid and NADH. NADH, under the action of PMS, transfer electrons to WST-8 to produce the yellow product, which has a characteristic absorption peak at 450 nm. Therefore, LDH activity can be quantified by measure the OD value at 450 nm.

5. Kit Components & Storage

Item	Specification	Storage
Lysis Solution	2 mL × 1 vial	-20°C, 6 months
Substrate	1.5 mL × 2 vials	-20°C, 6 months
Chromogenic Agent	1.5 mL × 2 vials	-20°C, 6 months, avoid direct sunlight
Enzyme Reagent	Lyophilized × 1 vial	-20°C, 6 months
Stop Solution	1.5 mL × 2 vials	-20°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Microplate centrifuge
- Water bath
- Microplate Reader (450 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- Double distilled water

6. Assay Notes:

1. According to the actual requirements, set different types of control wells.

2. Cells must be alive.

3. Remove air bubbles in the wells of the microplate when measuring the OD value.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use. Preheat stop solution at 37°C for 20 min in advance and can be used only after it is completely clarified.

2. Preparation of **enzyme working solution**: Dissolve a vial of enzyme reagent powder with 0.26 mL ultrapure water. Prepare fresh solution before use and can be stored at -20°C for 1 week.

3. Preparation of **reaction working solution**: Mix substrate, chromogenic agent, enzyme working solution at a ratio of 12:12:1. Prepare the needed fresh solution before use and store it by avoiding direct sunlight.

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 450 nm

9. Operation Steps

The preparation of sample

1. 96-well cell culture plates are added according to the following categories (each category with at least triplicate wells):

2. **Blank wells:** 100 µl of culture medium with no cells (It is recommended to use low-serum containing 1% serum or serum-free medium).
   Sample control wells: 100 µl of cells for detection (with 5-10 x 10^3 cells).
   High control wells: 100 µl of cells for detection (with 5-10 x 10^3 cells).
   Sample wells: 100 µl of cells for detection (with 5-10 x 10^3 cells).

3. Incubate cells for 24 h in an incubator (5% CO_2, 100% humidity, 37°C).

4. Add 10 µl of culture medium into blank wells and sample control wells.

5. Add 10 µl of drug stimulation with different concentrations into sample wells.

6. Incubate cells in an incubator (5% CO_2, 100% humidity, 37°C) (The incubation condition and time can be decreased or increased depend on the different cell).

7. Take out 96-well cell culture plates from the cell incubator before 1h at the end of culture, add 10 µl of lysis Solution into the high control wells , and beaten and mixed repeatedly.

8. Incubate cells for 1h in an incubator (5% CO_2, 100% humidity, 37°C).

9. Centrifuge cells at 400 g for 15 min in the microplate centrifuge and take the supernatant for detection.
   Note: If there is no microplate centrifuge, the cells can be transferred to the EP tube and centrifuged by ordinary centrifuge.

The measurement of samples

1. Prepare microplate and take 50 µl of supernatant into the corresponding blank, sample control, high control and sample wells.

2. Add 50 µl of reaction working solution to each well.

3. Add 50 µL of reaction working solution to each well and mix fully for 5 s with microplate reader

4. Incubate at 37°C for 10 min (The reaction time can be decreased or increased depend on the color development. The plate can be read at multiple time points until the desired reading is observed. The OD value of high control should be < 2.0, while the OD value of sample control should be < 0.8).

5. Add 20 µL of stop solution to each well, mix and stop the reaction.

6. Measure the OD values of each well at 405 nm with microplate reader. The reference wavelength should be 600 nm, which deducted is the required effective OD value

10. Calculations

$$\text{Cytotoxicity (\%)} = \frac{A_2 - A_1}{A_3 - A_1} \times 100\%$$

A_1: OD value of sample control well –OD value of blank well.
A_2: OD value of sample well –OD value of blank well.
A_3: OD value of high control well –OD value of blank well.

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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