PRODUCT INFORMATION & MANUAL

Lactate Dehydrogenase Assay Kit (Colorimetric)

NBP3-24513

For research use only.
Not for diagnostic or therapeutic procedures.

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Lactate Dehydrogenase Assay Kit (Colorimetric)

Catalog No: NBP3-24513

Method: Colorimetric method

Specification: 96T

Measuring instrument: Microplate reader

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Lysis Solution	2 mL × 1 vial	-20℃ , 12 months
Reagent 2	Substrate	1.5 mL × 2 vials	-20℃ , 12 months
Reagent 3	Chromogenic Agent	1.5 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Enzyme Reagent	Powder × 1 vial	-20℃ , 12 months
Reagent 5	Stop Solution	1.5 mL × 2 vials	-20℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲Materials prepared by users

Instruments

Microplate centrifuge, Micropipettor, Water bath, Microplate reader (450 nm).

Reagents

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key point of the assay

1. According to the actual requirements, set different types of control wells.
2. The cells must be alive.
3. There should be no bubbles in the wells of the microplate when measuring the OD value.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use. Preheat reagent 5 at 37°C for 20 min in advance and can be used only after it is completely clarified.
2. Preparation of reagent 4 working solution:
   Dissolve a vial of 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 reagent 2, reagent 3, reagent 4 working solution at a ratio of 12:12:1. Prepare the needed fresh solution before use and store it with shading light.

Assay protocol

▲Detailed operating steps

1.The preparation of sample

1) 96-well cell culture plates are added according to the following categories (each category with at least triplicate wells) :
   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);
2) Incubate cells for 24 h in an incubator (5% CO_2, 100% humidity, 37°C);
3) Add 10 μl of culture medium into blank wells and sample control wells;
   Add 10 μl of drug stimulation with different concentrations into sample wells;
4) 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);
5) Take out 96-well cell culture plates from the cell incubator before 1h at the end of culture, add 10 μl of reagent 1 into the high control wells , and beaten and mixed repeatedly;
6) Incubate cells for 1h in an incubator (5% CO_2, 100% humidity, 37°C);
7) 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.

2.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 and mix fully for 5 s with microplate reader;
3) 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);
4) Add 20 µL of reagent 5 to each well, mix and stop the reaction;
5) Measure the OD values of each well at 450 nm with microplate reader. The reference wavelength should be 600 nm, which deducted is the required effective OD value.

▲Calculation

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

Note:

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;

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