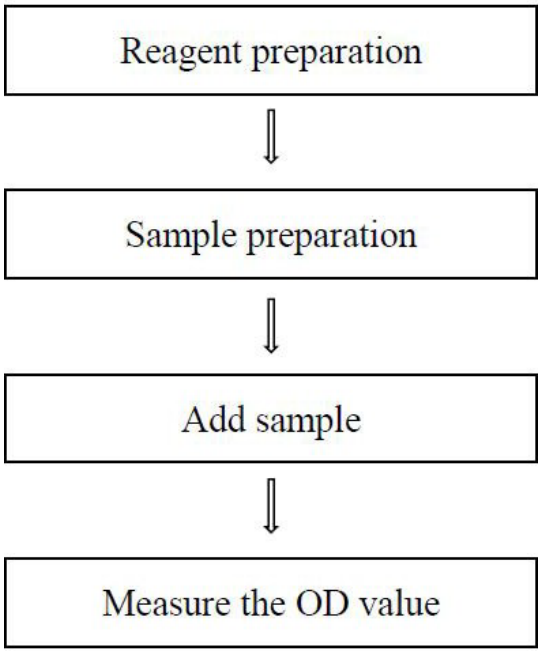
IMMUNOLOGICAL SCIENCES Product Data Sheet

IK-71001 Lactate Dehydrogenase (LDH) Cytotoxicity Colorimetric Assay Kit

Specification: 96T / 500Assays

Measuring instrument: Microplate reader（450 nm）

Assay summary



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Intended use

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

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	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Lysis Solution	2 mL × 1 vial	10 mL × 1 vial	-20℃, 12 months
Reagent 2	Substrate	1.5 mL × 2 vials	15 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Chromogenic Agent	1.5 mL × 2 vials	15 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Stop Solution	1.5 mL × 2 vials	15 mL × 1 vial	-20℃, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate 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 reaction working solution:

For each well, prepare 50 μL of reaction working solution (mix well 25 μL of substrate, 25 μL of chromogenic agent). The reaction working solution should be prepared on spot and stored protected from light.

The key points 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.

Operating 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) :
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 5-10×10^3 cells); High control wells: 100 μL of cells for detection (with 5-10×10^3 cells); Sample wells: 100 μL of cells for detection (with 5-10×10^3 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 1 h at the end of culture, add 10 μL of lysis solution into the high control wells , and beaten and mixed repeatedly.
⑥ Incubate cells for 1 h in an incubator (5% CO_2, 100% humidity, 37°C).
⑦ Centrifuge cells at 400×g for 5 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 sample

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℃ 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 stop solution 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.

Appendix I Example Analysis

Example analysis:

Detect SW480 cells and HCCLM3 cells (the concentration of protein is 4.40 gprot/L) according to the protocol, the result is as follows: