(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Lactate Dehydrogenase (LDH) Activity Assay Kit

Catalog No.: BC00003

Size: 100T/500T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Lactate Dehydrogenase (LDH) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, plasma, tissue, cells
Assay Type	Enzyme activity
Detection Instrument	Microplate reader (490nm. Reference wavelength for dual-wavelength determination: 600nm or any wavelength

Product Introduction

Lactate Dehydrogenase (LDH) Activity Assay Kit, also known as LDH Cytotoxicity Assay Kit or LDH Release Assay Kit. The destruction of cell membrane structure caused by cell apoptosis or necrosis will lead to the release of enzymes in the cytoplasm into the culture medium, including LDH with relatively stable enzyme activity. By detecting the activity of LDH released into the culture medium from cells with ruptured plasma membranes, quantitative analysis of cytotoxicity can be achieved. LDH release is regarded as an important indicator of cell membrane integrity and is widely used in cytotoxicity detection.

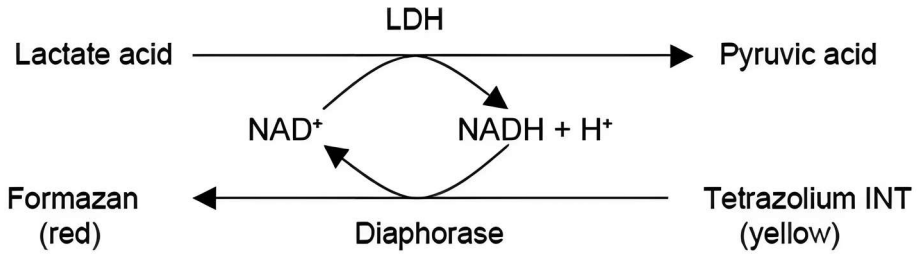
Product Features

- A wide range of detectable sample type: This assay kit is capable of detecting the activity of lactate dehydrogenase (LDH) in various samples such as cell culture supernatants and cell lysates. It is frequently used for cytotoxicity assays that take LDH release as an indicator. Moreover, based on the detection of total cellular LDH activity, this kit can also be used for the detection of cell proliferation and cytotoxicity.

Principle

Under the action of lactate dehydrogenase (LDH), NAD+ is reduced to generate NADH. NADH and INT (2-p-iodophenyl-3-nitrophenyl tetrazolium chloride) are then catalyzed by diaphorase to produce NAD+ and a strong-colored formazan. An absorption peak is generated at a wavelength of 490nm, which allows for the quantification of LDH activity through colorimetry. The absorbance is linearly and directly proportional to the activity of

lactate dehydrogenase. The schematic diagram of this enzyme-linked reaction principle is as follows:



Components

Components	Size (100T)	Size (500T)	Storage
LDH Release Reagent	1.5ml	7.5ml	-20℃
Lactic Acid Solution	1ml/vial, 2 vials	10ml	-20℃
Enzyme Solution	1ml/vial, 2 vials	10ml	-20°C, avoid repeated freeze-thaw cycles.
INT Solution (10×)	0.2ml	0.2ml	-20°C, protected from light.
INT Dilution Buffer	1ml/vial, 2 vials	1ml/ vial, 2 vials	-20℃
Microplate	1 plate	5 plates	RT
Plate Sealer	2 pieces	10 pieces	RT

Storage

The unopened kit can be stored at -20°C for 12 months. After thawing, the kit can be stored at 2-8°C for a short period of time and is valid within 2-3 days.

Preparation

- **Sample handling**

Method 1: LDH release detection

1. Inoculate an appropriate number of cells into a 96-well cell culture plate based on the size and growth rate of the cells to ensure that the cell density does not exceed 80-90% full at the time of testing.

2. Aspirate the culture medium and wash once with PBS. Replace with fresh culture medium (it is recommended to use low-serum culture medium containing 1% serum or appropriate serum-free culture medium), and divide each culture well into the following groups: including cell-free culture medium wells (background blank control wells), control cell wells without drug treatment (sample control wells), cell wells without drug treatment for subsequent lysis (sample maximum enzyme activity control wells), and drug-treated cell wells (drug-treated sample wells), and mark them well. Give appropriate drug treatment according to experimental needs (such as adding about 0-10μl of specific drug stimulation, different concentrations and different treatment times can be set, and appropriate drug solvent controls need to be added to the control wells), and continue to culture as usual. One hour before the scheduled detection time, take out the cell culture plate from the cell culture incubator and add the LDH release reagent provided by the kit to the "sample maximum enzyme activity control wells" at a volume of 10% of the original culture medium. After adding the LDH release reagent, mix well by pipetting up and down several times, and then continue to incubate in the cell culture incubator.
3. At the scheduled time, centrifuge the cell culture plate at 400g for 5 minutes using a multi-well plate centrifuge. Transfer 120μl of the supernatant from each well to a new 96-well plate in the corresponding wells, and proceed with the sample assay immediately.

Method 2: Detection of total intracellular LDH

1. Cytotoxicity assay:

Inoculate an appropriate number of cells into a 96-well cell culture plate based on the size and growth rate of the cells to ensure that the cell density does not exceed 80-90% full at the time of testing. Treat the cells with different drugs and set up appropriate controls. After the drug stimulation is complete, centrifuge the cell culture plate at 400g for 5 minutes using a multi-well plate centrifuge. Aspirate the supernatant as much as possible, then add 150μl of the LDH release reagent provided by the kit diluted 10 times with PBS (mix 1 volume of LDH release reagent with 10 volumes of PBS and mix well), gently shake the culture plate to mix, and then continue to incubate in the cell culture incubator for 1 hour. After incubation, centrifuge the cell culture plate again at 400g for 5 minutes using a multi-well plate centrifuge. Transfer 120μl of the supernatant from each well to a new 96-well plate in the corresponding wells, and proceed with the sample assay immediately.

2. **Cell proliferation assay:**
   Inoculate an appropriate number of cells into a 96-well cell culture plate based on the size and growth rate of the cells, ensuring that the cells do not exceed 80-90% full after stimulation with drugs that promote cell proliferation. Stimulate the cells with different drugs and set up appropriate controls. After the drug stimulation is complete, centrifuge the cell culture plate at 400g for 5 minutes using a multi-well plate centrifuge. Aspirate the supernatant as much as possible, then add 150μl of the LDH release reagent provided by the kit diluted 10 times with PBS (mix 1 volume of LDH release reagent with 10 volumes of PBS and mix well), gently shake the culture plate to mix, and then continue to incubate in the cell culture incubator for 1 hour. After incubation, centrifuge the cell culture plate again at 400g for 5 minutes using a multi-well plate centrifuge. Transfer 120μl of the supernatant from each well to a new 96-well plate in the corresponding wells, and proceed with the sample assay immediately.
   Note: The LDH release assay is more commonly used, and the total intracellular LDH assay can typically be replaced with methods such as MTT, WST-1, or CCK-8.

Method 3: Detection of LDH in samples

This kit can also be used for the detection of LDH in various samples. Sample requirements: Hemolysis should not be present in serum samples, as the LDH activity in red blood cells is about 100 times higher than that in serum; samples should not contain detergents such as SDS, Tween20, NP-40, or Triton X-100; oxalate anticoagulants should not be used.

1. Serum and plasma and other liquid samples: No pretreatment is required.
2. Tissue samples: The homogenization medium for tissue processing is physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). After homogenization, centrifuge at 4℃, 10000×g for 10 minutes, and take the supernatant to be measured on ice.
3. Cell samples: Take about 10^6 cells and add 300μL of physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) for mechanical homogenization or sonication. After homogenization, centrifuge at 4℃, 10000×g for 10 minutes, and take the supernatant to be measured on ice.
4. Before measurement, samples can be diluted with the LDH release reagent to two or three different concentrations to determine the dilution factor, ensuring that the LDH concentration is within the linear range of this kit (0-800 mU/ml). During the assay, take 120μl of the sample and add it to a 96-well plate for subsequent detection.

- **Preparation of the kit**

1. Preparation of INT Solution (1×): To prepare the required amount of INT solution (1×), dilute the appropriate amount of INT solution (10×) with INT diluent to 1× concentration. For example, take 20μl of INT solution (10×) and add 180μl of INT diluent, then mix well to prepare 200μl of INT solution (1×). The INT solution (1×) should be prepared fresh and used on the same day, stored at 4ºC, and should not be frozen after preparation.

2. Preparation of LDH Detection Working Solution: Based on the number of samples (including controls) to be tested, refer to the table below to freshly prepare an appropriate amount of detection working solution just before testing. Note: The LDH detection working solution must be prepared fresh and used immediately, and care should be taken to shield it from light during preparation and use.

Detections	1	10	20	50
Lactic Acid Solution	20μl	200μl	400μl	1ml
INT Solution (1×)	20μl	200μl	400μl	1ml
Enzyme Solution	20μl	200μl	400μl	1ml
Total Volume	60μl	600μl	1.2ml	3ml

3. (Optional) If you wish to perform absolute quantification of LDH enzyme activity, you will need to prepare LDH standards and freshly prepare LDH standards at different concentrations, such as 800 mU/ml, 400 mU/ml, 200 mU/ml, 100 mU/ml, and 0 mU/ml.

Operation process

1. Add 60μl of LDH detection working solution to each well.
2. Mix well and incubate at room temperature (approximately 25ºC) in the dark for 30 minutes (can be wrapped in aluminum foil and placed on a horizontal or reciprocating shaker for slow shaking). Then measure the absorbance at 490nm. Use a reference wavelength of 600nm or greater for dual-wavelength measurement.
3. Calculate (the absorbance of each group measured should be subtracted by the absorbance of the background blank control well).
4. Cytotoxicity or mortality (%) = (Absorbance of treated sample - Absorbance of sample control well) / (Absorbance of maximum cell enzyme activity - Absorbance of sample control well) × 100.
5. A cytotoxicity curve can be plotted: the vertical axis is the actual absorbance, and the

horizontal axis is the drug concentration; from this, the half-lethal dose (LD50) of the drug at a specific time can be calculated.

Calculation

Appendix 1

The absorbance values corresponding to a known concentration of LDH enzyme standards can be measured simultaneously. The activity of LDH enzyme in the sample can be roughly calculated using the following formula:

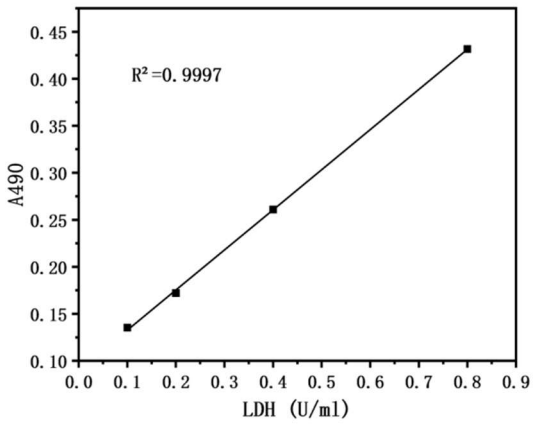
LDH activity units (mU/ml) in the sample = (Absorbance of the sample well - Absorbance of the background blank control well) / (Absorbance of the standard tube - Absorbance of the standard blank tube) × Concentration of the standard (mU/ml)

Based on the calculated results, statistical differences between different sample treatment groups can be compared.

Appendix 2

To accurately calculate the absolute activity of LDH enzyme activity, a standard curve can be plotted using a series of LDH standards and their corresponding measured absorbance values. The enzyme activity of LDH in the sample can then be calculated using the formula corresponding to the standard curve.

After subtracting the blank control from each well's value, plot the LDH standard curve with the detected absorbance (OD490) as the vertical axis and LDH enzyme activity (mU) as the horizontal axis. Also, calculate the formula for this trend line.



A490nm = k × LDH enzyme activity units (mU) + b, where the slope k and intercept b of the trend line are calculated using software such as Excel. The LDH activity in the sample is

calculated based on the above formula.
The actual absorbance of the sample (OD490) = the absorbance measured in the sample well - the absorbance of the background blank control well
LDH enzyme activity units (mU) in the detection system = (OD490 - b) / k
LDH enzyme activity (mU/ml) in the sample = LDH enzyme activity units (mU) in the detection system / volume of the tested sample.

Notes

1. Freezing can inactivate some lactate dehydrogenase (LDH) in the sample, and it can be stored at 4ºC for 2-3 days. It is recommended to complete the assay on the same day the samples are prepared.
2. If detecting LDH in cell culture supernatants, since serum contains LDH, it is recommended that the concentration of serum used should not exceed 1%, and heat-inactivated serum is preferred. If it is necessary to use 10% serum, a control well without cells but with an equal volume of culture medium should be included in the assay to eliminate the background.
3. Overgrowth of cells, high cell density, excessive centrifugation speed, and large temperature differences between the incubator and the outside environment can all lead to increased release of LDH from cells.
4. If absolute quantification of LDH activity is desired, users need to provide their own LDH standards.
5. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.