LDH Cytotoxicity Assay Kit

Product Information

Product name	Cat#	Size
LDH Cytotoxicity Assay Kit	40209ES76	500 T

Product Description

LDH Cytotoxicity Assay Kit, also known as LDH Assay Kit or LDH Release Assay Kit, The kit is a colorimetric assay for detecting lactate dehydrogenase activity released during cytotoxicity or in other samples based on diaphorase catalyzed INT chromogenic reaction.

Detection principle: LDH (lactate dehydrogenase) is rich in cytoplasm, can not pass through the cell membrane when normal, when the cell is injured or dead can be released to the outside of the cell, released LDH in the supernatant of culture medium, can be detected by enzyme reaction. In the presence of lactate dehydrogenase, NAD^+ was reduced to form NADH, and NADH and INT(a Tetrazolium salt) were catalyzed by Diaphorase to form NAD^+ and red dirty (Formazan). The amount of dirty was proportional to the number of lysed cells. The absorption peak was generated at 490 nm and the activity of lactate dehydrogenase could be quantified by colorimetry. There was a positive linear correlation between absorbance and lactate dehydrogenase activity.

LDH release is clearly a safer and more effective alternative to the ^{51}Cr labeling method followed by ^{51}Cr release for cell membrane integrity testing. This kit can be used for routine lactate dehydrogenase activity testing, more commonly used for LDH release as an indicator of cytotoxicity testing. The kit can also be used to detect cell proliferation and cytotoxicity based on total lactate dehydrogenase activity.

This kit can detect the activity of lactate dehydrogenase in cell culture medium, cell lysate and other samples. One kit can carry out 500 tests.

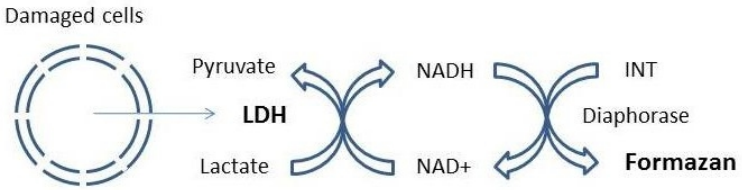

Figure :Schematic diagram of LDH Cytotoxicity Assay Kit

Product Components

Component number		12209ES24	12209ES96
40209-A	Cell Lysis Buffer	7.5 mL	-20℃
40209-B	Substrate Mix	10 mL	-20℃
40209-C	INT Substrate Solution (10×)	1 mL	-20℃， Avoid light
40209-D	INT Dilution Buffer	10 mL	-20℃
40209-E	Enzyme Solution	5 mL×2	-20℃， Avoid repeated freezing and thawing

Shipping and Storage

The components are shipped with ice pack and can be stored at -20°C for 1 year.

Cautions

1. It is recommended to complete the measurement on the same day as possible after the sample is ready. Freezing will inactivate part of the lactate dehydrogenase in the sample. It can be stored at 4℃ for 2-3 days.

2. As serum contains lactate dehydrogenase, it is recommended that the concentration of serum should not exceed 1%, and it is best to use heat-inactivated serum. If it is necessary to use 10% serum, a control well without cells but with the same volume of culture medium must be set up during testing to eliminate background.

3. Excessive cell growth, high density, excessive centrifugation speed, and large temperature difference between inside and outside the incubator will lead to increased lactate dehydrogenase release.

4. If absolute quantification of lactate dehydrogenase activity is desired, the lactate dehydrogenase standard should be provided.

5. Avoid bubbles during operation, as bubbles will affect the absorption value reading.

6. Avoid gentle operation when absorbing cells. Excessive strength will cause spontaneous LDH release and affect the reading.

7. For research use only!

Instructions

1. Sample preparation:

1. LDH release detection

① An appropriate amount of cells were inoculated into the 96-well cell culture plate according to the size and growth rate of cells, so that the cell density did not exceed 80-90% when being detected.

② The culture medium was sucked up and washed with PBS solution once. Replace fresh culture medium (it is recommended to use low-serum medium containing 1% serum or appropriate serum-free medium), and divide each culture well into the following groups: Cell-free culture medium Wells (background blank control well), untreated control cell Wells (sample control well), untreated cell Wells for subsequent lysis (sample maximum enzyme activity control well), and drug-treated cell Wells (drug-treated sample well) should be labeled. According to the experiment needs to give appropriate drug treatment (such as adding 0-10 μL or so specific drug stimulation, can set different concentrations, different treatment time, the control hole to add appropriate drug solvent control), continue to follow the conventional culture. One hour before the scheduled detection time, take out the cell culture plate from the cell culture box, and add the cell lysis solution provided by the kit into the control well for maximum enzyme activity of the sample", adding 10% of the volume of the original culture solution. After adding the cell lysate, repeatedly blow and mix it several times, and then continue to incubate in the cell incubator.

③ After reaching the predetermined time, centrifuge the cell culture plate with porous plate at 400 g for 5 min. Take 120 μL of supernatant from each well, add to corresponding well of a new 96-well plate, and then determine the sample.

2. Detection of intracellular total LDH：

① Cytotoxicity test: an appropriate amount of cells were inoculated into the 96-well cell culture plate according to the size and growth rate of cells, so that the cell density to be detected did not exceed 80-90%. Add different drugs for treatment, and set up appropriate control. After drug stimulation, the cell culture plate was centrifuged 400 g for 5 mins in a porous plate centrifuge. Absorb supernatant as much as possible, add 150 μL of cell lysis solution provided by 10 times of PBS (add 1 volume of cell lysis solution to 10 volumes of PBS and mix well), shake the culture plate properly and mix well, then continue to incubate in the cell incubator for 1 hour. Then the cell culture plate was centrifuged at 400 g for 5 mins in a porous plate centrifuge. All were taken 120 μL supernatant from the well was added to the corresponding well of a new 96-well plate, and then the sample was determined.

② Cell proliferation detection: according to the size and growth rate of cells, an appropriate amount of cells were inoculated into the 96-well cell culture plate, so that the cells stimulated by drugs promoting cell proliferation should not exceed 80-90%. Stimulate cells with different drugs and set appropriate controls. After drug stimulation, the cell culture plate was centrifuged 400g for 5 mins in a porous plate centrifuge. Absorb supernatant as much as possible, add 150 μL of cell lysis solution provided by 10 times of PBS (add 1 volume of cell lysis solution to 10 volumes of PBS and mix well), shake the mixture properly, then continue to incubate in cell incubator for 1 hour. Then the cell culture plate was centrifuged with a porous plate centrifuge of 400g 5 mins. Take 120 μL supernatant from each well, add to corresponding well of a new 96-well plate, and then determine the sample.

【Notes】： LDH release detection is more commonly used, and the detection of intracellular total LDH can usually be replaced by MTT, WST-1 or CCK-8.

2. **INT solution (1×) configuration:**

① INT solution (1×) configuration: according to the required amount of INT solution (1×), take an appropriate amount of INT solution (10×) diluted to 1× with INT diluent. For example, take 20 μL INT solution (10×), add 180 μL INT diluent, mix to make 200 μL INT solution (1×). INT solution (1×) should be prepared and used now, stored at 4℃ after configuration can be used on the same day, not frozen after configuration.

② Preparation of LDH detection working solution: according to the number of samples to be determined (including control), refer to the table below to prepare an appropriate amount of detection working solution before the detection.

[Note] : LDH detection working fluid must be prepared and used on the spot. Attention should be paid to avoiding light properly during preparation and use.

Testing number	1 test	10 test	20 test	50 test
Substrate Mix	20 μL	200 μL	400 μL	1 mL
INT Substrate Solution (1×)	20 μL	200 μL	400 μL	1 mL
Enzyme Solution	20 μL	200 μL	400 μL	1 mL
The total volume	60 μL	600 μL	1.2 mL	3 mL

③ (Optional): If you want to do the absolute quantification of LDH enzyme activity, you need to prepare your own LDH standard, and prepare different concentrations of LDH standard, such as 10mU/mL, 5 mU/mL, 2.5 mU/mL, 1.25 mU/mL, 0.65 mU/mL, 0 mU/mL.

Determination of the sample：

① Add 60 μL LDH detection solution to each well, respectively.

② Mix well, and incubate at room temperature (about 25℃) for 30 mins away from light (wrap it with aluminum foil and slowly shake it on a horizontal or side shaking shaker). The absorbance was then measured at 490 nm. Dual-wavelength measurements were performed using any wavelength at 600 nm or greater as a reference wavelength.

③ Calculation (the measured absorbance of each group should be subtracted from the absorbance of the background blank control hole).

Cytotoxicity or mortality (%)=(absorbance of treated sample - absorbance of sample control well)/(absorbance of maximum enzyme activity of cells - absorbance of sample control well)×100.

④ The cytotoxicity curve can be drawn: the vertical coordinate is the actual absorbance, the horizontal coordinate is the drug concentration; Based on this, the semi-lethal dose LD_{50} of the drug at a specific time of action can be calculated.

[Appendix 1 Relative quantification of LDH enzyme activity]:

The absorbance value corresponding to a standard LDH enzyme with a known concentration can be measured at the same time, and the LDH enzyme activity in the sample can be roughly calculated by referring to the following formula:

LDH activity unit (mU/mL) = (sample well OD_{490}- background blank control well OD_{490})/(standard tube OD_{490}- standard blank tube OD_{490})× standard concentration (mU/mL); According to the calculation results, the statistical difference between different sample treatment groups can be compared.

[Appendix 2 absolute quantification of LDH enzyme activity] :

In order to accurately calculate the absolute activity of LDH enzyme activity, a series of LDH standard products and the corresponding measured absorbance value can be used to draw the standard curve, and the corresponding formula of the standard curve can be used to calculate the LDH enzyme activity in the sample.

After the values of each well were subtracted from blank control, the standard CURVE of LDH was drawn using the detected absorbance (OD_{490}) as the ordinate and LDH enzyme activity (mU) as the abscissa. Meanwhile, the formula of the trend line is calculated.

A490nm = K ×LDH enzyme activity unit (mU)+b, the slope K and intercept B of the trend line were calculated by Excel and other software.

LDH activity in the sample was calculated according to the above formula.

Sample actual absorbance (OD_{490}) = sample well OD_{490}- background blank control well OD_{490}

LDH enzyme activity unit (mU)=(OD_{490}-B)/ K

LDH enzyme activity in the sample (mU/mL) = LDH enzyme activity unit in the detection system (mU)/ sample volume