LDH Cytotoxicity Assay Kit

Introduction

The LDH Cytotoxicity Assay Kit is a kit that assesses cytotoxicity by measuring the activity of lactate dehydrogenase (LDH) released into the culture medium. LDH is a stable enzyme found in all cells and is released from the cell into the medium when cells are damaged or apoptosis. LDH catalyzes the conversion of lactate to pyruvate, and this catalytic process reduces NAD^+ to NADH. The specific substrates provided in this kit can react with the resulting NADH, resulting in a product that is specifically absorbed at 490 nm. This specific absorption is directly proportional to the amount of LDH released into the culture medium Therefore, by measuring the change in absorbance, it is possible to indirectly assess the degree of cell damage or death.

Compared with the traditional ^{51}Cr cytotoxicity test, this kit contains no radioactive substances, which is simpler and safer to use. Moreover, the detection effect of the detection method used in this kit has the same effect as that of ^{51}Cr, and the results are reliable.

Components and Storage

Components	K2228-200T	K2228-1000T
Substrate Mix	1 vial	5 x 1 vial
Assay Buffer	1 mL	5 x 1 mL
Lysis Buffer (10X)	2.5 mL	12 mL
Stop Solution	12 mL	60 mL
LDH Positive Control	6 μL	30 μL
Store the kit at -20°C, stable for 1 year. Substrate Mix should be stored away from light.		

Protocol

1. **Preparation before the experiment:**

   1) Thaw and warm Assay Buffer, Lysis Buffer (10X), and Stop Solution to room temperature in advance.

   2) Prepare the substrate stock solution: Add 11 mL of ddH_2O to the Substrate Mix vial and mix gently to make the substrate stock solution.

***Note:** Protect from light when using Substrate Mix.

   3) Prepare the reaction working solution: Slowly add 1 mL of Assay Buffer to the substrate stock solution vial,

and mix well to prepare the reaction working solution. One vial of reaction working solution is sufficient to test two 96-well plates. The unused reaction solution can be stored at -20°C for 3-4 weeks in the dark, and the activity will not be affected within three freeze-thaw cycles.

***Note:** Protect from light when using and storing the reaction working solution.

4) Prepare 1X LDH Positive Control: Dilute 1.5 μL of LDH Positive Control with 1 mL of 1% BSA in PBS to make the 1X LDH Positive Control. Store unused LDH Positive Control at -20°C.

2. Pre-Experiment - Determine the optimal number of cells seeding for LDH cytotoxicity experiments

1) Seed two sets of cell suspensions (0-10000 cells/100 μL medium) in a series of concentration gradients in 96-well plates, and it is recommended to set 5-7 cell concentrations with three replicates of each concentration. One group is the LDH maximum release group and the other group is the LDH normal release group. Incubate the cells in a 37°C incubator overnight.

2) Add 10 μL of sterile water to each well of the LDH normal release group and mix gently. Add 10 μL of Lysis Buffer (10X) per well to the LDH maximum release group and mix gently.

***Note:** Be careful not to create bubbles.

3) Both groups of cells were incubated in a 37°C incubator for 45 minutes.

4) Transfer 50 μL of medium per well to a new 96-well plate.

5) (Optional) For LDH positive control experiments, add 50 μL of 1X LDH positive control into triplicate wells in the transferred new 96-well plate.

6) Add 50 μL of reaction working solution to each well and mix gently. The 96-well plate was incubated for 30 minutes at room temperature in the dark.

7) Add 50 μL of Stop Solution to each well and mix gently. Break any bubbles with a syringe needle or centrifugation before testing.

***Note:** After adding the Stop Solution, please test within 1-2 hours.

8) Absorbance value (A) is immediately detected at 490 nm. The absorbance at 680 nm is also detected as a reference (background value), and the absorbance value at 680 nm was deducted from the absorbance value at 490 nm to make the correction value (A') for subsequent analysis.

9) Plot ($A'_{\text{LDH maximum release group}} - A'_{\text{normal release group}}$) versus cell number to determine the linear range and optimal number of cells for LDH cytotoxicity assay.

3. LDH cytotoxicity assay

1) Seed the optimal number of cells/wells in 100 μL medium according to the pre-experiment in 96-well plates.

2) Incubate in a 37°C incubator overnight.

3) After incubation, treat cells according to the following table. Then incubate the plate in a 37°C incubator for a certain period. The specific time can be optimized depending on the interested drug.

LDH maximum release group	-
Negative control group	Add 10 μL of sterile water or drug solvent
Experimental group	Add 10 μL of the drug of interest

4) Add 10 μL of Lysis Buffer (10X) per well to the LDH maximum release group and gently mix.

***Note:** Be careful not to create bubbles.

5) Incubate in a 37°C incubator for 45 minutes.

6) Transfer 50 μL of medium per well to a new 96-well plate.

7) (Optional) For LDH positive control experiments, add 50 μL of 1X LDH positive control into triplicate wells in the transferred new 96-well plate.

8) Add 50 μL of reaction solution to each well and mix gently. The 96-well plate was incubated for 30 minutes at room temperature in the dark.

9) Add 50 μL of Stop Solution to each well and mix gently. Break any bubbles with a syringe needle or centrifugation before testing.

***Note:** After adding the Stop Solution, please test within 1-2 h.

10) Measure the absorbance at 490 nm and 680 nm.

11) Calculate cytotoxicity: Subtract the background absorbance at 680 nm from the absorbance of 490 nm to make the correction value A' for each well, and then calculate the cytotoxicity using the following formula:

Cell cytotoxicity%= $(A'_{\text{Experimental group}} - A'_{\text{Negative control group}})/(A'_{\text{LDH maximum release group}} - A'_{\text{Negative control group}}) \times 100\%$

Note

1. When cells are cultured for long periods, the corner and edge wells of the 96-well plate are prone to liquid evaporation. It is recommended to fill the surrounding moat with sterile water, medium, or PBS. Meanwhile, place the plate near the water source in the incubator.
2. The serum in the medium contains LDH, which may lead to a high background. To reduce the background, serum concentration can be lowered without affecting cell viability. When the serum concentration is reduced to 5%, the background value can be significantly reduced without affecting cell viability.
3. It is recommended to use a multichannel pipette during the experiment to reduce the difference between the multiple wells.
4. For your safety and health, please wear lab coats and gloves during the experiment.

5. For research use only. Not to be used in clinical diagnostic or clinical trials.

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