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Product overview

LDH-Cytotoxicity Assay Kit is based on using WST reagent for a fast and more sensitive detection of LDH released from damaged cells. The assay utilizing an enzymatic coupling reaction : LDH oxidizes lactate to generate NADH, which then reacts with WST to generate yellow color. The intensity of the generated color correlates directly with the cell number lysed. Since WST is brighter than other viability reagent, less amount of culture medium is required for the assay, and thus the background from serum and culture medium is significantly reduced. Cells can be cultured in regular 10% serum containing medium, no reducing serum or special medium is required for the assay. In addition, since the WST is very stable, the reaction can be read multiple times. LDH activity can be easily quantified by spectrophotometer or plate reader at OD450nm. The kit provides all necessary reagents including LDH positive control.

Kit Components

	100T	500T
WST Substrate Mix Solution	0.2ml	1ml
LDH Assay Buffer	10ml	50ml
Cell Lysis Solution	1ml	5ml

Preparation of Working Solutions

For 100 assays, mix 200µl of WST Substrate Mix Solution with 10.0 ml of LDH Assay Buffer. The LDH Reaction Mix should be stable for several weeks at 4°C.

Assay Protocol

1. Sample Preparation :

Collect cells (adherent or suspension) and wash once with fresh regular culture medium, then seed 100µl cells (with 2-10 x 10⁴ cells) in a 96-well plate as the following :

a. Background Control :

100µl culture medium per well in triplicates with no cells. The Background Control will measure reagents and LDH background from culture medium serum. The background value has to be subtracted from all other values.

b. Low Control :

100µl cells in triplicate wells

c. High Control :

100µl cells in triplicates, add 10µl Cell Lysis Solution each well, mix. To adjust the increase of medium volume, 11µl of the medium may be used in LDH activity assay at step 4.

d. Test Sample:

100µl cells in triplicates, add test substances each well, mix.

*** Notes:**

- a) Trypsin may be used to remove adherent cells from a culture surface before seeding in a 96-well plate.
- b) The amount of cells to be used per well depends on the cell types. To optimize the assay, you can do a quick testing by using 2, 4, 8 x 10⁴ cells per well, and then follow the assay protocol to determine the cell number you should use. The high control should be OD450nm ~2.0 after 30 min treatment with 10% Cell Lysis Solution, while the low control should be OD450nm < 0.8. The reaction time should be set at ~ 30 min.
- c) If the test substances are not dissolved in PBS, a solvent control may be performed by addition of the same amount of solvent in triplicates without testing substances.

2. Sample Incubation: Incubate cells in an incubator (5% CO₂, 90% humidity, 37°C) for the appropriate time of treatment determined for test substance. Gently shake the plate at end of the incubation to ensure LDH is evenly distributed in the culture medium.

3. Centrifuge cells at 600 x g for 10 min to precipitate the cells.

4. Transfer the clear medium solution (10µl/well) into an optically clear 96-well plate.

5. LDH Reaction Mix: The solution is stable for two month at 4°C. Add 100µl LDH Reaction Mix to each well, mix and incubate for 30 min at room temperature.

6. Measure the absorbance of all controls and samples with a plate reader equipped with 450 nm (440 - 490 nm) filter. The reference wavelength should be 650 nm.

***Notes:**

- a) The reaction time can be decreased or increased depending on the color development. The plate can be read at multiple time points until the desired reading is observed. The high control should be OD450nm ~2.0, while the low control should be OD450nm < 0.8.

Data Analysis

$$\text{Cytotoxicity (\%)} = \frac{(\text{Test Sample} - \text{Low Control})}{(\text{High Control} - \text{Low Control})} \times 100$$

Store condition

-20°C

For Research Use Only. Not For Use In Diagnostic Procedures.