Technical Bulletin

LDH Cytotoxicity Assay Kit II

Catalog Number MAK380

Product Description

Cell death (cytotoxicity) is classically evaluated by the quantification of plasma membrane damage. Lactate dehydrogenase (LDH) is a stable enzyme, present in all cell types, and rapidly released upon damage to the plasma membrane. LDH, therefore, is the most widely used marker in cytotoxicity studies.

The LDH Cytotoxicity Assay Kit II utilizes and advanced WST (Water Soluble Tetrazolium salts) for the fast and sensitive detection of LDH released from damaged cells. The assay utilizes an enzymatic coupling reaction: LDH oxidizes lactate to generate NADH, which then reacts with WST to produce a yellow color. The intensity of the generated color correlates directly with the number of cells lysed. Since the advanced WST is brighter than traditional WST, less amount of culture medium is required for the assay, and the background from serum and culture medium is significantly reduced. Using the assay, cells can be cultured in media containing 10% serum; no reducing serum or special medium is required for the assay. In addition, since the WST color generation is stable, the reaction can be read multiple times and be stopped at any time point during the reaction. LDH activity can be easily quantified by spectrophotometer or plate reader at 450 nm. The kit provides all the necessary reagents including LDH positive control, and the assay time is less than 1 hour.

The kit is suitable for the determination of Lactate dehydrogenase (LDH) activity in cells.

Components

The kit is sufficient for 500 colorimetric assays in 96-well plates.

- WST Substrate Mix — 1 vial
  Catalog Number MAK380A
- LDH Assay Buffer — 50 mL
  Catalog Number MAK380B
- Cell Lysis Solution — 5 mL
  Catalog Number MAK380C
- Stop Solution — 5 mL
  Catalog Number MAK380D
- LDH (Positive Control) — 1 vial
  Catalog Number MAK380E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96 well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Incubator (5% CO_2, 90% humidity, 37 °C)
- Centrifuge capable of RCF ≥ 600 × *g*

Precautions and Disclaimer

For R&D Use Only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

WST Substrate Mix: Reconstitute vial with 1.1 mL of purified water. Mix thoroughly for 10 minutes. The solution is stable for two months at 2-8 °C.

LDH (Positive Control): Reconstitute vial with 100 µL of LDH Assay Buffer.

LDH Reaction Mix: For each 100 assays, mix 200 µL of WST Substrate Mix with 10 mL of LDH Assay Buffer.

Procedure

Sample Preparation

1. Collect cells (adherent or suspension) and wash once with fresh regular culture medium, then seed 100 µL of cells (with 2-10 × 10^4 cells*) in a 96-well plate per the following:

   Background Control: Add 100 µL of culture medium (no cells) per well in triplicate. The Background Control will measure reagents and LDH background from culture medium serum. The background value must be subtracted from all other values.

   Low Control: Add 100 µL of cells per well in triplicate.

   High Control: Add 100 µL of cells per well in triplicate, then add 10 µL of Cell Lysis Solution to each well and mix. To adjust the increase of medium volume, 11 µL of the medium may be used in the LDH activity assay at Step 4.

   Test Sample: Add 100 µL of cells per well in triplicate, then add test substances to each well and mix.

Notes:

a) Trypsin may be used to remove adherent cells from a culture surface before seeding in a 96-well plate.

b) The number of cells to be used per well depends on the cell type. To optimize the assay, perform a quick test by using 2, 4, and 8 × 10^4 cells per well, and then follow the assay protocol to determine the cell number to use. The High Control A_{450} reading should be ~ 2.0 after the 30 minute treatment with 10% Cell Lysis Solution, while the Low Control A_{450} reading should be < 0.8. The reaction time should be set at ~30 minutes.

c) Positive Control (5 µL LDH) can be used to test whether all reagents are working properly in response to active LDH enzyme.

d) If the test substances are not dissolved in PBS, a solvent control may be performed by addition of the same amount of solvent in triplicate without testing substances.

2. Incubate cells in an incubator (5% CO_2, 90% humidity, 37 °C) for the appropriate time of treatment determined for the test substance. Gently shake the plate at the end of the incubation to ensure LDH is evenly distributed in the culture medium.
3. Centrifuge cells at 600 × *g* for 10 minutes to precipitate the cells.

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

Assay Procedure

1. Add 100 µL of LDH Reaction Mix to each well, mix and incubate for 30 minutes** at room temperature.
2. Measure the absorbance of all controls and samples at 450 nm (A_{450}).

Notes:

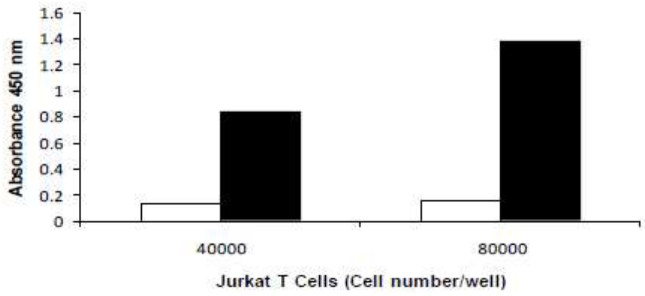
a) ** The reaction time can be decreased or increased depending on the color development. The plate can be read at multiple time points until the desired reading is observed. The High Control should be A_{450} ~2.0, while the Low Control should be A_{450} < 0.8.

b) The reaction can be stopped by adding 10 µL of Stop Solution and mixing. The reaction may be read within 48 hours without significant changes. Protect the reaction from light and evaporation.

Results

$$\text{Cytotoxicity (\%)} = \frac{(A_{450\ \text{test sample}}) - (A_{450\ \text{Low Control}})}{(A_{450\ \text{High Control}}) - (A_{450\ \text{Low Control}})} \times 100$$

Figure 1.
LDH Cytotoxicity Assay Kit II. Jurkat T cells were cultured in a 96-well plate in 100 µL of culture medium. LDH Assay was performed using 10 µL of culture medium using the WST probe. Low Control (white bar); High control (black bar).



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