LDH-Cytotoxicity Colorimetric Assay Kit II

(Catalog# K313-500, 500 assays; Store kit at -20°C)

I. Introduction:

Cell death or 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 into the cell culture medium upon damage of the plasma membrane. LDH, therefore, is the most widely used marker in cytotoxicity study. **BioVision's LDH Cytotoxicity Assay Kit II** utilizes the advanced WST reagent for a fast and more sensitive detection of LDH released from the damaged cells. The assay utilizes 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, less amount of culture medium is required for the assay, and thus the background from serum and culture medium is significantly reduced. Using the assay, 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 more stable, the reaction can be read multiple times, and can also be stopped at any time point during the reaction. LDH activity can be easily quantified by spectrophotometer or plate reader at OD 450 nm. The kit provides all the necessary reagents including LDH positive control. The assay takes less than 1 hour.

II. Kit Contents:

Component	K313-500 500 assays	Cap Color	Part Number
WST Substrate Mix	1 Bottle	Amber	K313-500-1
LDH Assay Buffer	50 ml	NM	K313-500-2
Cell Lysis Solution	5 ml	Clear/NM	K313-500-3
Stop Solution	5 ml	Blue	K313-500-4
LDH (Positive Control)	Lyophilized	Red	K313-500-5

III. Preparation of Working Solutions:

- Reconstitute the **WST Substrate Mix** in 1.1 ml ddH_2O for 10 min and mix thoroughly. The solution is stable for two month at 4°C.
- Reconstitute **LDH Positive Control** with 100 µl of LDH Assay Buffer.
- Preparation of **LDH Reaction Mix:** For 100 assays, mix 200 µl of WST Substrate Mix with 10.0 ml of LDH Assay Buffer. The LDH Reaction Mix should be stable for several weeks at 4°C.

VI. LDH-Cytotoxicity Assay Protocol:

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

   Background Control: 100 µl 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.

   Low Control: 100 µl cells in triplicate wells.

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

   Test Sample: 100 µl 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 10^4 cells per well, and then follow the assay protocol to determine the cell number you should use. The High Control should be $OD_{450\ nm}$ ~ 2.0 after 30 min treatment with 10% Cell Lysis Solution, while the low control should be $OD_{450\ nm}$ < 0.8. The reaction time should be set at ~ 30 min.

   c) Positive Control (5 µl LDH) can be used to test whether all reagents are working properly to 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 triplicates without testing substances.

2. Incubate cells in an incubator (5% CO_2, 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 (10 µl/well) into an optically clear 96-well plate.
5. Add 100 µl 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.

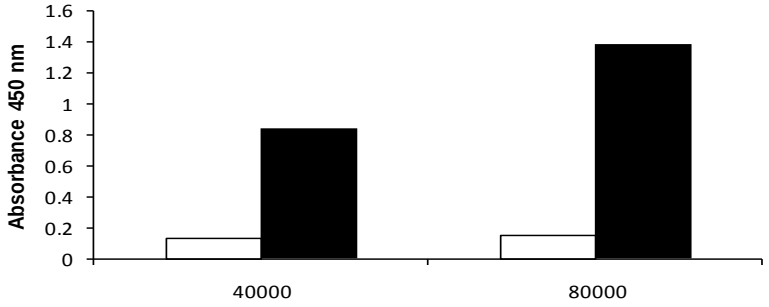
****Notes:**

a) The reaction time can be decreased or increased depend on the color development. The plate can be read at multiple time points until the desired reading is observed. The high control should be $OD_{450\ nm}$ ~ 2.0, while the low control should be $OD_{450\ nm}$ < 0.8.

b) The reaction can be stopped by adding 10 µl of Stop Solution, mix and read within 48 hr without significant changes. Protect the reaction from light and evaporation.

V. Calculation of the Percentage Cytotoxicity:

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



LDH Cytotoxicity Assay Kit II. Jurkat T cells were cultured in 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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