LDH-Cytotoxicity Colorimetric Assay Kit

(Catalog # K311-400; 400 assays; Store kit at –20˚C)

I. Introduction:

Cell death or cytotoxicity is classically evaluated by the quantification of plasma membrane damage. **The LDH-Cytotoxicity Assay Kit** provides a fast and simple method for quantitating cytotoxicity based on the measurement of activity of lactate dehydrogenase (LDH) released from damaged cells. Unlike many other cytoplasmic enzymes which exist in many cells either in low amount (e.g., alkaline and acid phosphatase) or unstable, LDH is a stable cytoplasmic enzyme present in all cells and rapidly released into the cell culture supernatant upon damage of the plasma membrane. LDH activity can be determined by a coupled enzymatic reaction: LDH oxidizes lactate to pyruvate which then reacts with tetrazolium salt INT to form formazan. The increase in the amount of formazan produced in culture supernatant directly correlates to the increase in the number of lysed cells. The formazan dye is water-soluble and can be detected by spectrophotometer at 500 nm. The LDH-cytotoxicity assay is sensitive, convenient, and precise, and is applicable to a variety of cytotoxicity studies. Assay takes ~0.5 – 1 hr.

II. Kit Contents:

Component	K311-400 400 assays	Part Number
Catalyst (lyophilized)	1 vial	K311-400-1
Dye Solution	45 ml	K311-400-2

III. Preparation of Working Solutions:

- Reconstitute the Catalyst in 1 ml ddH_2O for 10 min and mix thoroughly. The Catalyst Solution is stable for several weeks at 4˚C.
- After thaw, the Dye Solution is stable for several weeks at 4˚C. Avoid freeze/thaw cycles.
- Preparation of Reaction Mixture: For 100 assays, mix 250 µl of Catalyst Solution with 11.25 ml of Dye Solution. The mixture solution should be prepared immediately before use.

IV. LDH-Cytotoxicity Assay Protocol:

1. Collect cells (adherent or suspension) and wash 1X with assay medium (e.g., medium containing 1 % serum or 1 % BSA).
   Note: Trypsin may be used to remove adherent cells from a culture surface.
2. Preparing the following samples individually in a 96-well plate:
   Background Control: Add 200 µl medium/well into triplicate wells. The background value has to be subtracted from all other values.
   Low Control: Add 1-2 x 10^4 cells/well in 200 µl assay medium into triplicate wells.
   High Control: Add 1-2 x 10^4 cells/well in 200 µl assay medium containing 1% Triton X-100 into triplicate wells.
   Test Sample: Add 1 - 2 x 10^4 cells/well in 200 µl assay medium containing test substance into triplicate wells.
3. Incubate cells in an incubator (5 % CO_2, 90 % humidity, 37˚C) for the appropriate time of treatment determined for test substance.
4. Centrifuge the cells at 250 g for 10 min.
5. Transfer 100 µl/well supernatant carefully into corresponding wells of an optically clear 96-well plate.
6. Add 100 µl Reaction Mixture to each well and incubate for up to 30 min at room temperature. Protect the plate from light.
7. Measure the absorbance of all samples at 490 - 500 nm using a microtiter plate reader. The reference wavelength should be more than 600 nm.

V. Calculation of the Percentage Cytotoxicity:

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

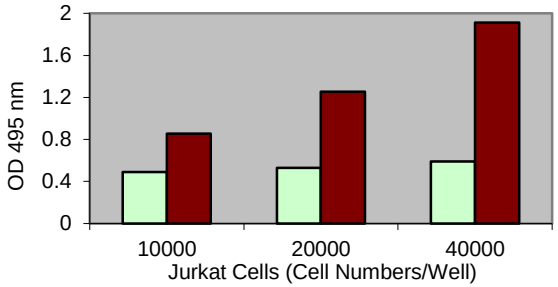

Fig. 1. Jurkat cells were cultured in 96-well plate in 100 µl of culture medium. LDH assay was performed using 10 µl of culture medium according to the kit instructions. Light bar: Low control; Dark bar: High control.

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