Lactate Dehydrogenase-Cytotoxicity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1323

(Version 1.2B)

Detection and Quantification of cytotoxicity based on Lactate Dehydrogenase (LDH) released from damaged cells.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Cell death or cytotoxicity is evaluated by the quantification of plasma membrane Damage. The damage to cell membrane structures results in cytoplasmic enzymes being released into the culture medium. In these enzymes, Lactate Dehydrogenase (LDH) is a stable cytoplasmic enzyme present in all cells and rapidly released into the cell culture supernatant upon damage of the plasma membrane.

Lactate Dehydrogenase-Cytotoxicity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring lactate dehydrogenase activity released from damaged cells. In this colorimetric LDH quantification assay, LDH reduces NAD to NADH, which then interacts with a specific probe to produce a color. The rate of increase in the absorbency at 490 nm, is a measure of LDH activity.

II. KIT COMPONENTS

Component	100 Assays	500 Assays	Storage
Lysis Buffer	4 ml x 1	20 ml x 1	4 °C
Reaction Buffer	15 ml x 1	15 ml x 5	-20 °C, keep in dark, avoid freeze-thaw cycles
Substrate Mix	Powder x 1	Powder x 5	-20 °C, keep in dark
Positive Control Diluent	1.2 ml x 1	1.2 ml x 1	4 °C
Positive Control	Powder x 1	Powder x 1	-20 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Incubator (5 % CO_2, 90 % humidity, 37 °C)
2. Inverted optical microscope
3. PBS
4. Cell culture medium containing 1% serum or 1% BSA
5. Microplate reader to read absorbance at 490 nm
6. Distilled water
7. Pipettor, multi-channel pipettor
8. Pipette tips
9. 96- or 384-well culture plates
10. Ice
11. Centrifuge
12. Timer

IV. REAGENT PREPARATION

Working Solution: Thaw 1 vial of Reaction Buffer and briefly centrifuge 1 vial of Substrate Mix prior to opening. For 100 assays, add 15 ml Reaction Buffer to a vial of Substrate Mix to generate Working Solution. Prepare Working Solution for immediate use. Store the unused solution in dark at 4°C for 3 days or -20 °C for 1 week, avoid freeze-thaw cycles.

Positive Control: Briefly centrifuge prior to opening. Dissolve in 1 ml Positive Control Diluent to generate stock solution. Dilute the stock solution 20-fold using Positive Control Diluent to prepare the Positive Control working solution (eg. 10 µl to 190 µl Positive Control Diluent). Store at -80 °C for 1 month.

V. SAMPLE PREPARATION

1. Set up 96-well assay plates containing cells in culture medium. Maintain cells at ≤80-90% confluency to avoid overgrowth.
2. Aspirate the culture medium, wash once with PBS, and replace with Assay Medium (medium with 1 % serum or 1 % BSA). Culture medium can lead to higher background signals for LDH in serum.
3. Perform the following samples and controls individually in 96-well plate:

   Background Control: Add 200 µl assay medium.

   Only Cells Control: Add untreated cells in 200 µl assay medium.

   Maximum LDH Control (Optional): Add 20 µl Lysis Solution in 200 µl untreated cells and continue to culture 1 hour before centrifugation.

   Test Sample: Add the test substance (cytotoxicity or proliferation assays) in untreated cells.
4. Incubate cells at 37 ° C for the appropriate time of treatment determined for test substance*.
5. Centrifuge the cells at 400 g for 5 min. Transfer 50 µl aliquots from all test and control wells to microplate.

*LDH has a half-life of approximately 9 hours when released into cell culture media. The incubation time will have to be optimized.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Test Sample*	Background Control	Only Cells Control	Maximum LDH Control	Positive Control
Sample	50 µl	--	--	--	--
Background Control	--	50 µl	--	--	--
Only Cells Control	--	--	50 µl	--	--
Maximum LDH Control	--	--	--	50 µl	--
Positive Control	--	--	--	--	50 µl
Working Solution	150 µl	150 µl	150 µl	150 µl	150 µl
Mix, incubate at room temperature for 5 minutes, record absorbance measured at 490 nm.					

Note:

***** Various cell types contain different amounts of LDH. For unknown samples, we recommend doing a pilot experiment to determine the optimum number of cells to use.

VII. CALCULATION

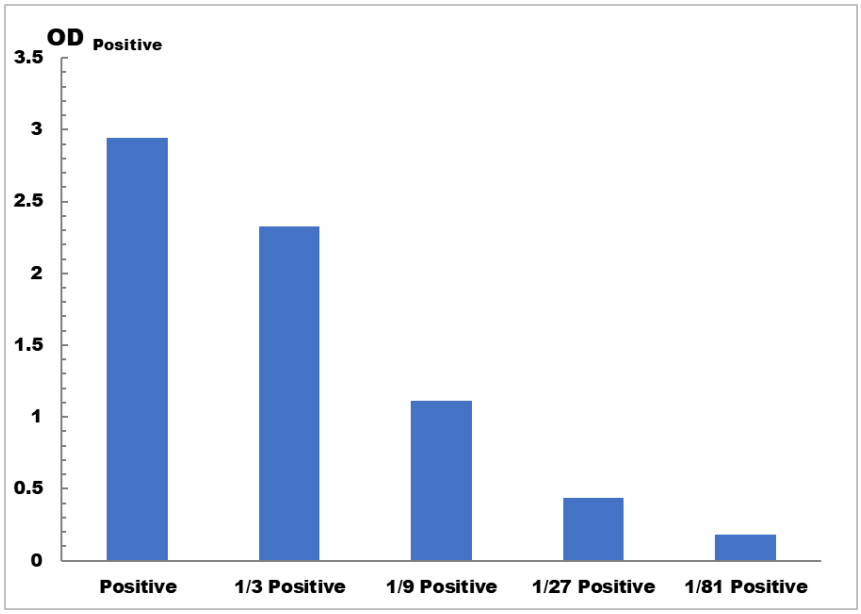
1. Calculation of the Cytotoxicity Percentage:

$$\text{Cytotoxicity (\%)} = \frac{OD_{\text{Test sample}} - OD_{\text{Only cells control}}}{OD_{\text{Maximum LDH control}} - OD_{\text{Only cells control}}} \times 100$$

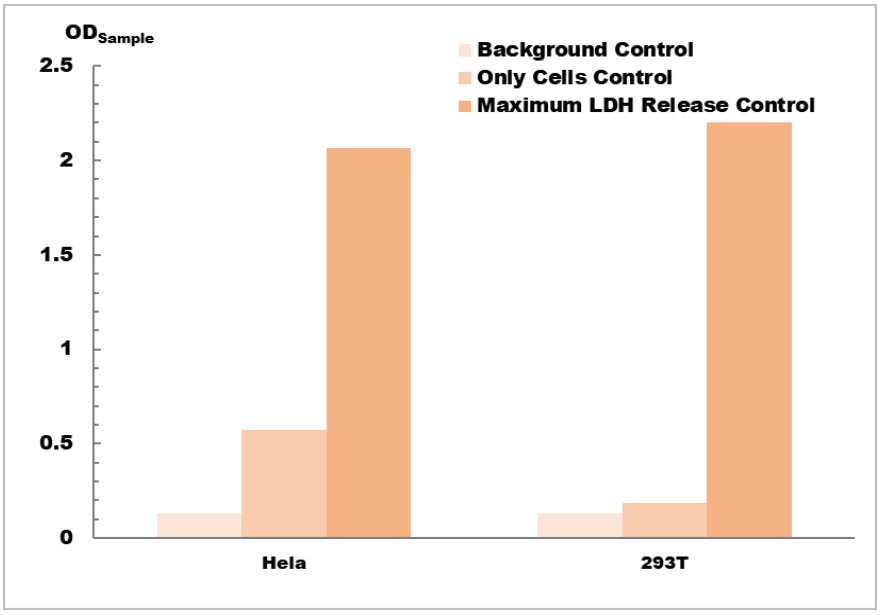
2. Plot the Cytotoxicity Curve:

Generate a cytotoxicity curve with $OD_{\text{Test sample}} - OD_{\text{Only cells control}}$ as y-axis versus drug concentration as x-axis, from which the LD_{50} at the designated time can be derived.

VIII. TYPICAL DATA



Positive Control reaction in 96-well plate assay with decreasing the concentration



Determination of LDH activity in cell lines with different amounts of LDH