ELISA PRODUCT INFORMATION & MANUAL

LDH-Cytotoxicity Assay Kit (Colorimetric)
(Colorimetric)

NBP2-54847

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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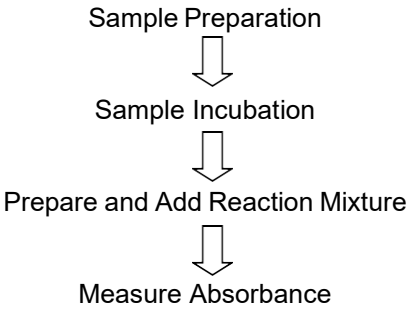
1. Overview

Cell death or cytotoxicity is classically evaluated by the quantification of plasma membrane damage. Novus Biologicals's LDH-Cytotoxicity Assay Kit (Colorimetric) 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Catalyst (Lyophilized)	1 vial
Dye Solution	45 mL

* Store kit at -20°C

CATALYST SOLUTION: 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.

DYE SOLUTION: After thawing, the Dye Solution is stable for several weeks at +4°C. Avoid freeze/thaw cycles.

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.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Collect cells (adherent and 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. Prepare the following samples individually in a 96-well plate:

a. Background Control:
Add 200 µl medium/well into triplicate wells. The background value has to be subtracted from all other values.

b. Low Control:
Add 1-2 x 10^4 cells/well in 200 µl assay medium into triplicate wells.

c. High Control:
Add 1-2 x 10^4 cells/well in 200 µl assay medium containing 1% Triton X-100 into triplicate wells.

d. 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 x g for 10 min.

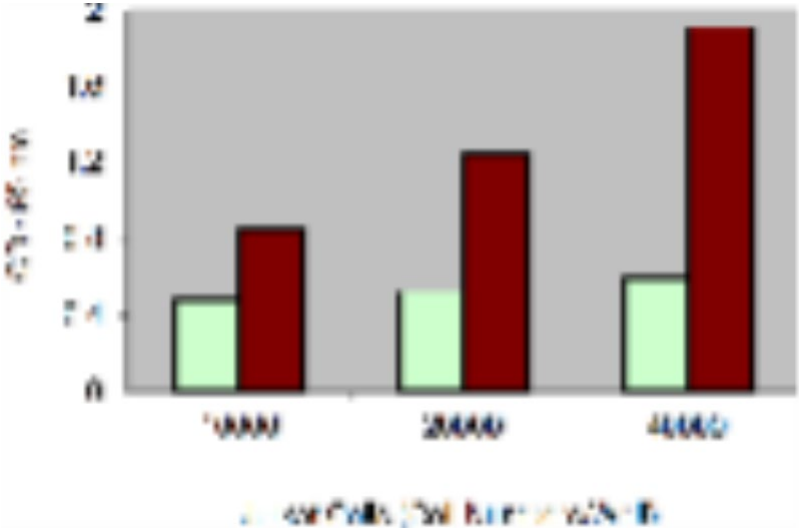
5. Transfer 100 µl/well supernatant carefully into corresponding wells of an optically clear 96-well plate.

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.

5. Data Analysis

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



Light bar: Low control

Dark bar: High control.

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.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Re-check datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit