LDH Cytotoxicity Fluorometric Assay Kit (BN00581)

(Catalog BN00581; 500 assays; Store at -20°C)

I. Introduction:

Lactate Dehydrogenase (LDH, EC 1.1.1.27) is a stable enzyme and is present in all cell types. Cell damage leads to release of LDH enzyme into the media and its activity is widely used as a marker for cytotoxicity. It reversibly converts lactate into pyruvate, with the concomitant interconversion of NADH and NAD^+. LDH Cytotoxicity Fluorometric Assay Kit provides a sensitive, quick, and easy way for detection of LDH released from damaged cells. In this assay, LDH converts lactate to pyruvate and NADH, which reduces a proprietary probe to an intensely fluorescent product (Ex/Em = 535/587 nm). The amount of fluorescence is directly proportional to the number of damaged cells. The assay is adaptable to high-throughput format and can be completed in less than 20 min. Sensitivity: ~ 100 cells.

$$\text{Lactate + NAD} \xrightarrow{\text{LDH}} \text{Pyruvate + NADH} \xrightarrow{\text{Probe}} \text{Fluorescence (Ex/Em = 535/587 nm)}$$

II. Applications:

- Measurement of released LDH in culture medium
- Evaluation of cytotoxicity effect of test compounds

III. Sample Type:

- Adherent or Suspension Cells

IV. Kit Contents:

Components	BN00581	Cap Color
LDH Assay Buffer	50 ml	NM
LDH Substrate Mix	lyophilized	Orange
Probe	2 ml	Amber
Cell Lysis Solution	5 ml	Clear
LDH Positive Control	1 vial	Red

V. User Supplied Reagents and Equipment:

- 96-well tissue culture plate with flat bottom
- 96-well white plate with flat bottom for fluorometric measurement
- Multichannel pipette
- Multi-well spectrophotometer (A fluorescent plate reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Read entire protocol before performing the assay. Briefly centrifuge small vials prior to opening.

- **LDH Assay Buffer:** Bring to room temperature before use. Store at -20°C or 4°C.
- **LDH Substrate Mix:** Reconstitute with 1.1 ml dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.
- **Probe:** Ready to use as supplied. Thaw and warm to room temperature before use. Store at -20°C. Use within two months.
- **LDH Positive Control:** Reconstitute LDH Positive Control with 200 µl LDH Assay Buffer. Add 2-5 µl reconstituted LDH as Positive Control Keep on ice when using.

VII. LDH Cytotoxicity Assay Protocol:

1. **Sample Preparation:** Collect cells (adherent or suspension) and wash once with fresh regular culture medium. Seed an appropriate number of cells in 100 µl culture medium per well of 96-well tissue culture plate for Low Control, High Control and to evaluate test compounds. Prepare parallel well(s) as medium background control and add 100 µl culture medium without cells. Incubate cells in an incubator (5% CO_2, 90% humidity, 37°C) overnight. Add test compounds to test compounds wells and incubate for an appropriate time based on the compound (~8-24 hrs). Shake plate for 1 min. to mix. At the end of incubation, gently shake the plate to ensure LDH is evenly distributed in the medium. In High Control well(s), add 10 µl Cell Lysis Solution/well, shake plate for 1 min. to mix, and incubate at 37°C for 30 min. Add 5 µl of supernatant from each well into a new 96-well white plate with flat bottom. In case of High Control well(s), 5.5 µl of medium may be used to adjust the increase of medium volume.

   Notes:

   a. The number of cells to be used per well depends on the cell type. To optimize the assay, perform a serial dilution starting with $2X10^4$ cells/well, then follow the protocol to determine the optimal cell numbers for the cytotoxicity assay.

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

   c. Medium Background control measures LDH background from reagents and culture medium. Animal serum in culture medium contains detectable amount of LDH. During analysis, the background value should be subtracted from all readings.

   d. Diluted LDH positive control (2-10 µl) can be used to ensure that all reagents are working properly.

   e. If the test compounds are not dissolved in PBS, a solvent control should be performed by addition of same amount of solvent to the medium as the test compound well.

   f. If suspension cells are used, centrifuge cells at 600 X g for 10 min. prior to collection of supernatant.

2. **LDH Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 95 µl of LDH Reaction Mix containing:

LDH Reaction Mix

LDH Substrate Mix	2 µl
Probe	4 µl
LDH Assay Buffer	89 µl

Mix well and add 95 µl of LDH Reaction Mix into each well. Mix and wrap with foil.

Note: Prepare LDH reaction mix just before use. Don't store the LDH Reaction Mix.

3. **Measurement:** Gently shake the plate for 10 min. at room temperature. After 10 min. incubation, measure fluorescence (Ex/Em = 535/587 nm).

   Note: The incubation time can be decreased or increased depending on the fluorescence signal. The plate can be read at multiple time points until the desired reading is observed.

4. **Calculation:** If medium background control reading is significant, subtract the medium background control reading from all readings. Calculate the percentage cytotoxicity as following:

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

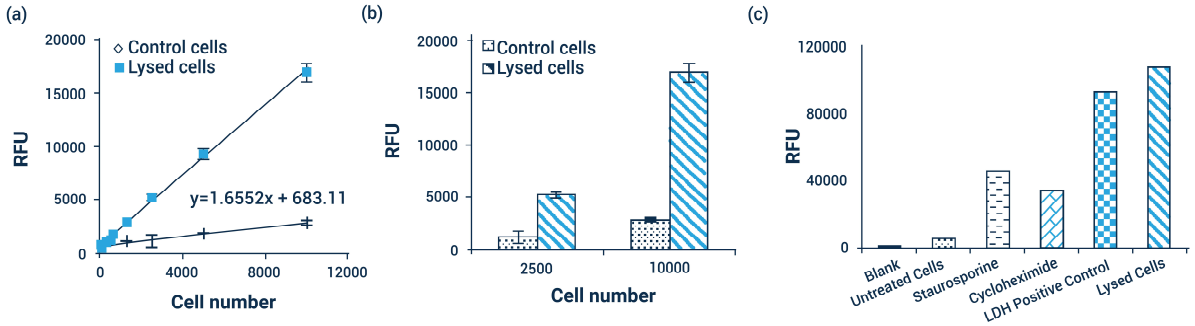

Figure: (a) Untreated Jurkat cells (low control) or treated with Cell Lysis Solution for 30 min. (high control), **(b)** Relative fluorescence units of untreated Jurkat cells (low control) or treated with Cell Lysis Solution for 30 min. (high control), **(c)** Overnight treatment of HeLa cells with 100 µM of staurosporine or 3 µM of cycloheximide. LDH released into the medium was measured along with blank, untreated cells (low control), LDH Positive Control, and lysed cells (high control). Assay was performed according to the kit protocol.

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