ELISA PRODUCT INFORMATION & MANUAL

LDH-Cytotoxicity Assay Kit (Fluorometric)

NBP2-54851

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

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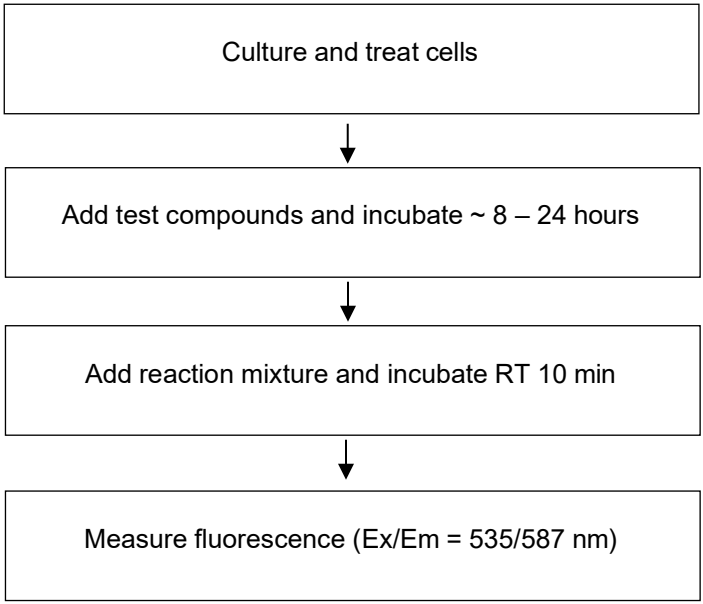
1. BACKGROUND

LDH-Cytotoxicity Assay Kit (Fluorometric) (NBP2-54851) provides a sensitive, quick, and easy way to detect LDH (lactate dehydrogenase) 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.

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+.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
LDH Assay Buffer	50 mL	-20°C	4°C / -20°C
LDH Substrate Mix	1 vial	-20°C	-20°C
Picoprobe	2 mL	-20°C	-20°C
Cell Lysis Solution	5 mL	-20°C	-20°C
LDH Positive Control	1 vial	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips – preferably a multichannel pipette
- Fluorescent microplate reader – equipped with filter for Ex/Em = 535/587 nm
- 96 well plate: white plates (clear flat bottoms) for fluorometric assay
- Heat block or water bath
- Trypsin (if using adherent cells)
- Appropriate cell culture medium
- 37°C cell incubator (5% CO_2, 90% humidity)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **LDH Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C or 4°C.

9.2 **LDH Substrate Mix:**

Reconstitute in 1.1 mL ddH_2O. Pipette up and down to dissolve completely. Aliquot substrate so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.3 **PicoProbe:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C protected from light. Once the probe is thawed, use within two months.

9.4 **LDH Positive Control:**

Reconstitute LDH Positive Control with 200 µl LDH Assay Buffer. Aliquot standard so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

10. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- 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 (96wp plate), then follow the protocol to determine the optimal cell numbers for the cytotoxicity assay.

10.1 Cells (adherent or suspension) cells:

10.1.1 Wash cells in PBS.

Use trypsin to release adherent cells from culture surface.

10.1.2 Collect cells and wash once with fresh regular culture medium.

10.1.3 Count cells.

10.1.4 Seed an appropriate number of cells in 100 µL culture medium per well of 96-well tissue culture plate.

Set up the following wells:

- Negative Control
- Lysate Control
- Medium Background Control: to measure LDH background from reagents and culture medium. Animal serum contains detectable amount of LDH.
- Solvent Control: to evaluate the effect of the compounds solvent in cells if test compounds are not dissolved in PBS.
- Test samples: to evaluate cytotoxic test compounds.

10.1.5 Incubate cells in an incubator at 37°C (5% CO2, 90% humidity) overnight.

10.1.6 Add test compounds to appropriate wells. Shake plate for 1 minute to mix. Incubate for the appropriate time for the compound of choice (8 – 24 hours).

10.1.7 Lysate Control wells only: prior to harvesting cells, add 10 µL Cell Lysis Solution/well, shake plate for 1 minute to mix and incubate at 37°C for 30 minutes.

10.1.8 At the end of incubation, gently shake the plate to ensure LDH is evenly distributed in the medium.

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**
- **Diluted LDH positive control (2-10 µL) can be used to ensure that all reagents are working properly.**

11.1 **Harvest culture medium**:

For adherent cells, collect medium from culture well plate. Centrifuge medium briefly to discard any cell debris.

For suspension cells, centrifuge cells at 600 x *g* for 10 minutes prior to collection of supernatant.

11.2 Pipette **5 µL of supernatant** from each well into a new 96-well white plate with flat bottom.

For Lysate Control wells, 5.5 µL of medium may be used to adjust the increase of medium volume.

11.3 LDH Positive Control: add 2 – 5 µL of diluted LDH positive control (Section 9.4) to culture medium in an empty well in the 96-well white plate with flat bottom.

11.4 **LDH Reaction Mix:**

Prepare 95 µL of Reaction Mix for each reaction immediately prior use:

Component	Reaction Mix (µL)
LDH Substrate Mix	**2**
Picoprobe	**4**
LDH Assay Buffer	**89**

Mix enough reagents for the number of assays (test samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + controls +1).

11.5 Add 95 µL of Reaction Mix into each test sample, negative control, lysate control, medium background control, positive control and solvent control (if using) wells.

11.6 Mix and wrap with foil.

11.7 Gently shake the plate at room temperature for 10 minutes.

11.8 Measure fluorescence on a microplate reader at 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*.

12. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12.1 Average the duplicate reading for each control and sample.

12.2 If the medium background control is significant, then subtract the sample background control from sample reading.

12.3 Calculate the percentage cytotoxicity as follows:

$$Cytotoxicity\,\% = \left(\frac{(Test\ Sample - Neg\ Control)}{(Lysate\ Control - Neg\ Control)}\right) x\ 100$$

13. TYPICAL DATA

Data provided for **demonstration purposes only**.

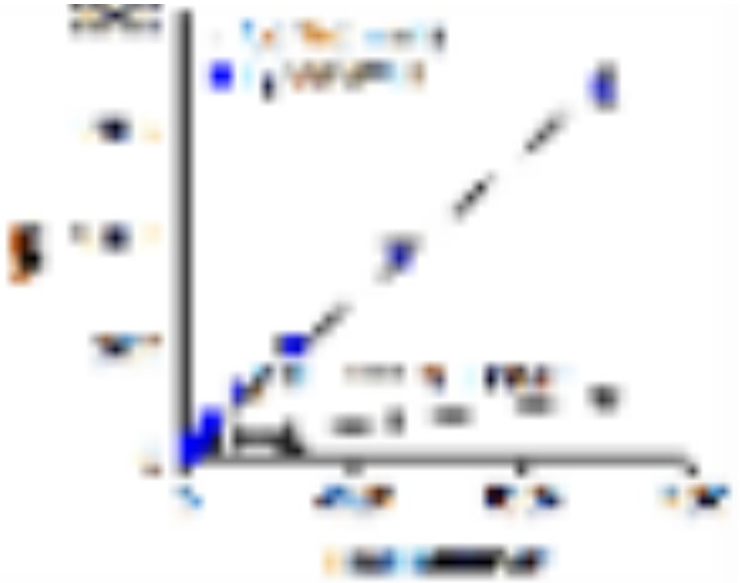


Figure 1. Untreated Jurkat cells (Negative Control) or treated with Cell Lysis Solution for 30 minutes, (Lysate Control).

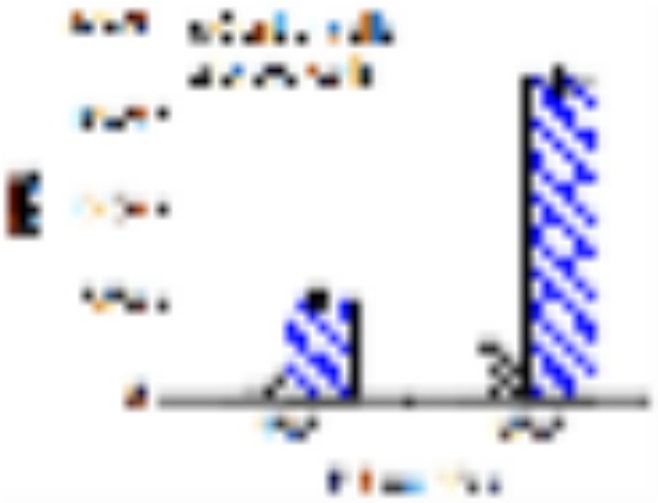


Figure 2: Relative fluorescence units of untreated Jurkat cells (Negative Control) or treated with Cell Lysis Solution for 30 minutes (Lysate Control).

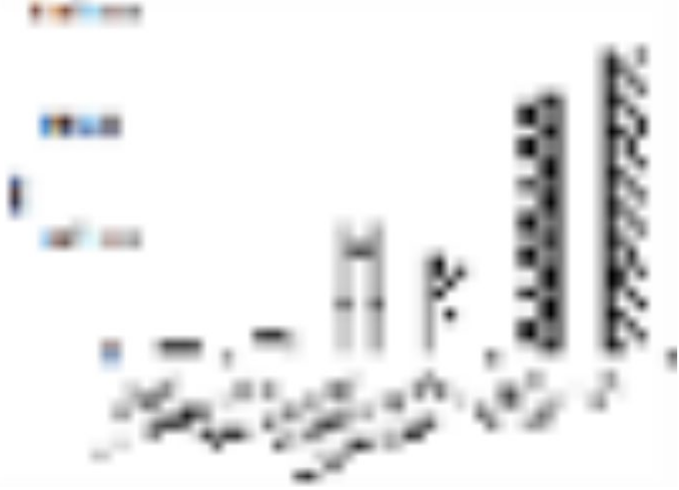


Figure 3: Overnight treatment of HeLa cells with 100 µM of staurosporine or 3 µM of cycloheximide. LDH released into the medium was measured along with blank, untreated cells (Negative Control), LDH Positive Control, and lysed cells (Lysate Control).

14. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare assay buffer, substrate mix, probe and positive control; (aliquot if necessary); get equipment ready.
- Seed an appropriate number of cells in 100 µL culture medium per well of 96-well tissue culture plate for Negative Control, Lysate Control and for test compounds.
- Medium Background Control = 100 µL culture medium without cells.
- LDH Positive Control = 100 µL culture medium + 2 – 10 µL diluted LDH positive control.
- Incubate cells in an incubator (5% CO_2, 90% humidity, 37°C) overnight.
- Add test compounds to appropriate wells and incubate for the appropriate determined time
- At the end of incubation, gently shake the plate.
- In Lysate Control wells, add 10 µL Cell Lysis Solution/well.
- Shake plate for 1 min to mix, and incubate at 37°C 30 mins.
- Add 5 µL of supernatant from each well into a new 96-well white plate with flat bottom. (for Lysate Control well(s), 5.5 µL of medium may be used to adjust the increase of medium volume).
- Prepare LDH Reaction Mix (Number samples + controls + 1).

Component	Reaction Mix (µL)
LDH Substrate Mix	**2**
Picoprobe	**4**
LDH Assay Buffer	**89**

- Add 95 µL of LDH Reaction Mix to the standard, sample, positive control and background control sample wells.
- Mix and wrap plate with foil.
- Gently shake plate RT 10 minutes.
- Measure plate at Ex/Em= 535/587 on a fluorescent microplate reader.

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16. **FAQ**

17. **INTERFERENCES**

18. NOTES