Store at -20°C

LDH Cytotoxicity Assay Kit

#37291

1 Kit
(480 assays)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

New 04/21

For Research Use Only. Not For Use In Diagnostic Procedures.

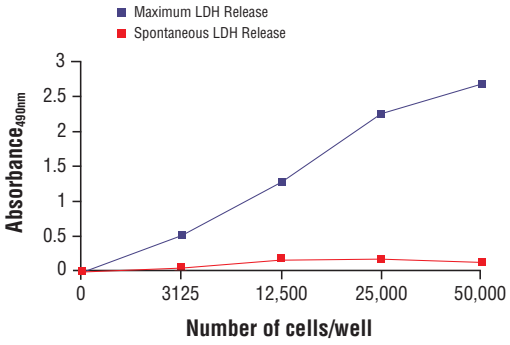
Products Included	Item #	Quantity	Storage Temperature
Diaphorase	50173	1 x 1 each	-20°C
INT (100X)	34743	1 x 500 µl	-20°C
LDH Positive Control	67843	1 x 1 each	-20°C
Lactic Acid (100X)	23346	1 x 500 µl	4°C
NAD^+ (100X)	95899	1 x 500 µl	4°C
Triton™ X-100 (10%)	86488	1 x 10 ml	RT
Cell-Based Assay Buffer Tablet	27868	1 x 1 each	RT

Description: The LDH Cytotoxicity Assay Kit can be used for quantitatively measuring cytotoxicity in response to chemical compounds as well as assaying cell-mediated cytotoxicity using a coupled two-step, colorimetric reaction. In the first step, LDH catalyzes the reduction of NAD+ to NADH and H+ by oxidation of lactate to pyruvate. In the second step of the reaction, diaphorase uses the newly-formed NADH and H+ to catalyze the reduction of a tetrazolium salt (INT) to highly-colored formazan, which absorbs strongly at 490-520 nm. The amount of formazan produced is proportional to the amount of LDH released into the culture medium as a result of cytotoxicity.

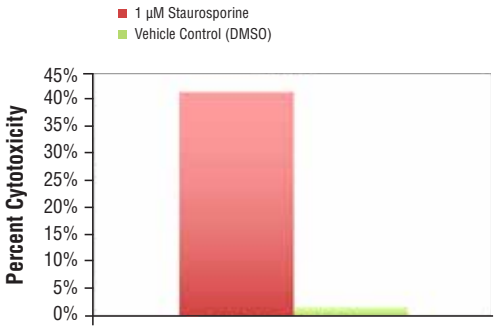
In a prototypical cytotoxicity assay, target cells are cultured with a cytotoxic chemical agent or a cytotoxic cell (e.g., NK cells) to induce target cell death and LDH release. The LDH-containing supernatants are transferred to wells of a new assay plate and mixed with the LDH Reaction Solution. After an incubation of 30 minutes at room temperature, the absorbance at 490 nm (A490) is read using a plate reader. Cells treated with cytotoxic agents or cytotoxic cells will release an amount of LDH that falls between the maximum release control level and the spontaneous release control level.

Background: Cell death can occur either by apoptosis, a highly regulated biochemical pathway involving signal transduction cascades, or by necrosis. Necrosis is accompanied by mitochondrial swelling and increased plasma membrane permeability, while apoptosis involves an articulated breakdown of the cell into membrane-bound apoptotic bodies (1). There are a number of assays that are designed to measure cytotoxicity and cell death, independent of mechanism. Most of these assays assess cell viability by measuring plasma membrane permeability (2).

Lactate dehydrogenase (LDH) is a stable, soluble enzyme located in the cytosol of many different cell types. The enzyme is released into the surrounding culture medium upon plasma membrane damage. LDH activity in the culture medium can, therefore, be used as a reliable indicator of cell membrane integrity, and thus a measurement of cytotoxicity.



Determination of Optimum Cell Number for LDH Cytotoxicity Assay. *HeLa cells were seeded into a 96-well plate at varying densities using media containing 10% FBS. After overnight incubation, the cells were replaced with serum-free media and then treated with either Assay Buffer (Spontaneous LDH Release) or 10% Triton™ X-100 solution (Maximum LDH Release). After treatment, the medium was removed and placed into a new 96-well plate. The amount of LDH released into the medium was determined using the LDH Cytotoxicity Assay Kit protocol.*



Determination of Staurosporine-induced LDH Release. *HeLa cells were seeded in medium supplemented with 1% FBS at density of 10,000 cells/well and then treated for 24 hr with either vehicle control or 1 µM Staurosporine #9953. LDH released into the medium and percent cytotoxicity was calculated using the LDH Cytotoxicity Assay Kit protocol.*

Storage: All components in this kit are stable for at least 12 months when stored at the recommended temperature.

Background References:

(1) Bonfoco, E. et al. (1995) *Proc Natl Acad Sci U S A* 92, 7162-6.

(2) Haslam, G. et al. (2000) *Cytotechnology* 32, 63-75.

Thank you for your recent purchase. If you would like to provide a review visit cellsignal.com/comments.

www.cellsignal.com

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

LDH Cytotoxicity Assay Kit Protocol

A. Solutions and Reagents

Supplied Reagents

1. **Diaphorase (#50173):** Reconstitute with 600ul of RODI water. (Keep on ice during use. Store at -20°C when not in use. Freezing and thawing of this solution should be limited to a single time. If you plan to use this solution in multiple experiments, we recommend that you aliquot it for storage at -20°C.)
2. **NAD^+ (100X):** #95899
3. **Lactic Acid (100X):** #23346
4. **INT (100X):** #34743
5. **LDH Positive Control (#67843); optional:** The LDH Positive Control in solution is very temperature-sensitive. Immediately prior to use, reconstitute in 1.8ml ice-cold RODI water. Do not vortex, mix gently. Maintain reconstituted LDH Positive Control on ice while performing the assay. Aliquot and immediately freeze unused reconstituted LDH Positive Control at -80°C. Frozen aliquots must be thawed on ice and thawed only once.
6. **Cell-Based Assay Buffer Tablet (#27868):** Dissolve in 100ml of RODI water. (This buffer should be stable for approximately one year at room temperature.)
7. **Triton™ X-100 (10%):** #86488

LDH Reaction Solution

To make 10ml of LDH Reaction Solution, sufficient for use on one 96-well plate, add 100ul of the following to 9.6ml of assay buffer:

- NAD^+ (100X): #95899
- Lactic Acid (100X): #23346
- INT (100X): #34743
- Reconstituted Diaphorase

Any leftover LDH Reaction Solution should be discarded after use, as it is not stable. If less than a full 96-well plate is to be used in an experiment, adjust the volumes of each of the reactants accordingly. Store remaining **INT (100X)** at -20°C. Store **NAD^+ (100X)** and **lactic acid (100X)** at 4°C.

Additional Reagents (Not Supplied)

1. Plate reader capable of measuring absorbance between 490-520 nm.
2. A plate centrifuge
3. Orbital Shaker
4. 96-well assay plate

B. Assay Specific Considerations

Note: Serum used to supplement growth medium (fetal calf serum, etc.) contains LDH that will react with the LDH Reaction Solution and induce a "background" color change (A490), even in the absence of cell death. The higher the percentage of serum in the medium, the higher the background signal will be. There are two solutions to this background problem; grow the cells in the absence of serum, or subtract the background signal from all wells prior to calculation of % cytotoxicity. Removal of serum from a growth medium can have negative impact on overall cell viability, so this may not be an option for all cell types. Subtraction of the background signal is easier, requiring simply the addition of wells to the assay that contain medium-only, without added cells. The LDH A490 signal resulting from the medium-only controls can be subtracted from all test wells after reading the plate.

Note: Different cell types contain different amounts of LDH. For cells with high LDH levels, fewer cells per well will be required to produce a strong A490 value than for cells with relatively low LDH levels. Therefore, we recommend performing an initial titration experiment to determine the optimal number of cells per well of the target cell you plan to use.

ASSAY PROTOCOL

Determination of optimal target cell number and growth medium

1. Seed target cells in a 96-well plate at a density of 10^3-10^6 cells/well in 200ul of culture medium. Prepare six wells at each cell concentration.
2. Add 200ul of medium only (without cells) to three wells (background control).
3. Add 20ul of Triton™ X-100 (10%) (#86488) to three wells, and 20ul of Assay Buffer to three wells, and incubate at room temperature for one hour.
4. Centrifuge the 96-well tissue culture plate at 400 x g for five minutes (optional but recommended).
5. Transfer 100ul of cell supernatant to a new 96-well assay plate.
6. Add 100ul of LDH Reaction Solution to each well. (*Remove bubbles from each well that are created during this step*)
7. Incubate the plate with gentle shaking on an orbital shaker for 30 minutes at 37°C protected from light.
8. Read the absorbance at 490 nm with a plate reader.
9. Repeat the assay above with culture medium containing low serum (0-2%) and high serum (10-20%) to assess the effects of serum on cell viability (spontaneous release) and background, or include extra wells in the initial assay to accommodate this variable.

Performing a Cytotoxicity Assay

1. Seed target cells in a 96-well plate at the previously determined optimal density in 200ul of culture medium.
2. Add 200ul of medium only (without cells) to three wells for background control, and to three wells for LDH Positive Control (**optional**).
3. Add 20ul of Triton™ X-100 (10%) (#86488) solution to three wells containing cells (maximum release) and 20ul of Assay buffer to three wells (spontaneous release). Add 20ul of the LDH Positive Control to three wells (optional).
4. Add 20ul of experimental cytotoxic agent to appropriate wells in triplicate.
5. Incubate the plate in a CO_2 incubator at 37°C for the length of time required by your experiment to induce cytotoxicity.
6. Centrifuge the 96-well tissue culture plate at 400 x g for five minutes (optional but recommended).
7. Transfer 100ul of cell supernatant to a new 96-well assay plate.
8. Add 100ul of LDH Reaction Solution to each well. (*Remove bubbles from each well that are created during this step*)
9. Incubate the plate with gentle shaking on an orbital shaker for 30 minutes at 37°C protected from light.
10. Read the absorbance at 490 nm with a plate reader.
11. Subtract background A490 levels from all wells.
12. Calculate % Cytotoxicity as follows:

$$\left[\frac{\text{(Experimental Value A490) – (Spontaneous Release A490)}}{\text{(Maximum Release A490 – (Spontaneous Release A490)}}\right] \times 100$$