LDH Cytotoxicity Assay Kit

Catalog No. 10008882

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Catalog Number	Item	96 wells Quantity/Size	480 wells Quantity/Size	Storage
10009318	LDH Diaphorase	1 vial	1 vial	-20°C
10009319	LDH NAD^+ (100X)	1 vial/120 µl	1 vial/550 µl	4°C
10009320	LDH Lactic Acid (100X)	1 vial/120 µl	1 vial/550 µl	4°C
10009328	LDH INT (100X)	1 vial/120 µl	1 vial/550 µl	-20°C
10009321	LDH Standard	1 vial	1 vial	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 tablet	1 tablet	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.
For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at the temperatures outlined in **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 490-520 nm
2. Adjustable pipettes and a repeat pipettor
3. A 96-well plate for culturing cells
4. A plate centrifuge
5. Distilled water

INTRODUCTION

Background

Cell death can occur either by apoptosis, a highly regulated 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 screening techniques available that detect 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 soluble enzyme located in the cytosol. The enzyme is released into the surrounding culture medium upon cell damage or lysis, processes that occur during both apoptosis and necrosis. LDH activity in the culture medium can, therefore, be used as an indicator of cell membrane integrity, and thus a measurement of cytotoxicity. Since the activity of intracellular LDH corresponds to the number of cells in the culture, quantification of LDH in cell lysates can be used as a measurement of cell growth.[2,3]

About This Assay

Cayman's LDH Cytotoxicity Assay Kit measures cell death in response to chemical compounds or environmental factors using a coupled two-step 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.

PRE-ASSAY PREPARATION

Cell Culture Preparation

1. Seed cells in a 96-well plate at a density of 10^4-10^5 cells/well in 120 µl of culture medium with or without compounds to be tested. We recommend that each treatment be performed in triplicate.
2. Culture the cells in a CO_2 incubator at 37°C for 24-48 hours.

Reagent Preparation

Some of the kit components are in lyophilized or concentrated form and need to be reconstituted or diluted prior to use. Follow the directions carefully to ensure proper volumes of LDH Assay Buffer are used to reconstitute or dilute the vial components.

1. Assay Buffer Preparation

Dissolve the Cell-Based Assay Buffer Tablet (Catalog No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. LDH Diaphorase

1 each vial LDH Diaphorase (96-well kit; Catalog No. 10009318): Reconstitute with 150 µl Assay Buffer.

OR

1 each vial LDH Diaphorase (480-well kit; Catalog No. 10009318): Reconstitute with 600 µl Assay Buffer.

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.

3. LDH Reaction Solution

To make 10 ml of Reaction Solution, sufficient for use on one 96-well plate, add 100 µl of the following to 9.6 ml of the Assay Buffer:

NAD^+ (100X) (Catalog No. 10009319)

Lactic Acid (100X) (Catalog No. 10009320)

INT (100X) (Catalog No. 10009328)

Reconstituted diaphorase (prepared in Step 2)

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

NOTE: Fetal bovine serum (FBS) contains LDH and thus will interfere with the results of this assay. We recommend that you use culture medium containing a low percentage of FBS (such as 1%) or serum free culture medium for this assay. When culture medium containing a high percentage of FBS (such as 10%) is used, wells containing this same medium should be included in the assay as a control.

LDH Standard

Immediately prior to use, reconstitute the contents of the LDH Standard (Catalog No. 10009321) in 1.8 ml of Assay Buffer. The standard should be kept on ice during use. This standard is unstable, and degrades rapidly if not stored at -80°C. If you plan to use the standard in multiple experiments, we recommend that you aliquot the reconstituted solution and store at -80°C. The reconstituted standard should be frozen and thawed only once.

To prepare the standard for use in the LDH assay: obtain six clean test tubes and label them #1 through #6. Aliquot 475 µl of culture medium into tube #1 and 250 µl into tubes #2-#6. Transfer 25 µl of the reconstituted LDH standard into tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, is 10 mU/ml (10,000 µU/ml). Serially dilute the standard by removing 250 µl from tube #1 and place into tube #2; mix thoroughly. Next, remove 250 µl from tube #2 and place into tube #3; mix thoroughly. Repeat this procedure for tubes #4 and #5. Do not add any standard to tube #6. This tube will be your blank.

NOTE: 1 Unit (U) is the amount of LDH that catalyzes the reaction of 1 µmol of substrate per minute.

Plate Set Up

Each plate should contain a standard curve, wells without cells, and wells containing cells with experimental treatment or vehicle. We recommend that standards be run in duplicate and that each treatment be performed in triplicate. A suggested plate format is shown below in Figure 1. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest that you record the contents of each well on the template sheet provided (see page 15).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	13	13	13	21	21	21
B	S1	S2	S3	S4	S5	S6	14	14	14	22	22	22
C	1	1	1	7	7	7	15	15	15	23	23	23
D	2	2	2	8	8	8	16	16	16	24	24	24
E	3	3	3	9	9	9	17	17	17	25	25	25
F	4	4	4	10	10	10	18	18	18	26	26	26
G	5	5	5	11	11	11	19	19	19	27	27	27
H	6	6	6	12	12	12	20	20	20	28	28	28

S1-S6 - Standards 1-6
1-28 - Samples

Figure 1. Sample plate format

Performing the Assay

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

NOTE: If you anticipate a high rate of cell death and you are planning to use the LDH standard to calculate the level of cytotoxicity, serial dilution of your samples may be required to obtain values that fall on the standard curve.

Procedure

1. Centrifuge the 96-well tissue culture plate at 400 x g for five minutes.
2. Using a new 96-well plate, transfer 100 µl of the standards prepared above into the appropriate wells. We recommend that the standards be run in duplicate.
3. Transfer 100 µl of each supernatant from each well of the cultured cells to corresponding wells on the new plate.
4. Add 100 µl of Reaction Solution (prepared in Step 3) to each well using a repeating pipettor.
5. Incubate the plate with gentle shaking on an orbital shaker for 30 minutes at room temperature.
6. Read the absorbance at 490 nm with a plate reader.

ANALYSIS

Calculations

Subtract the Blanks

Average the absorbance value of the wells containing assay buffer medium only (standard #6) and subtract this from the absorbance values of all the other wells.

Plot the Standard Curve

Make a plot of absorbance at 490 nm as a function of LDH concentration and determine the equation of the line. See figure 2, on page 11, for a typical standard curve.

Determine the Sample Concentration

Determination of LDH activity present in the sample

$$\text{LDH Activity (µU)} = \frac{(A_{490\ nm} - \text{y-intercept})}{\text{slope}}$$

$$\text{Total LDH Activity (µU/ml) in sample} = \frac{\text{Value from LDH activity assay (µU)}}{\text{x sample volume assayed (usually 0.1 ml)}}$$

Sensitivity:

The assay can detect as few as 10^3 cells, depending on cell type.

Performance Characteristics

Sample Data

The standard curve presented here is an example of the data typically produced in culture medium RPMI 1640 without FBS. However, your results will not be identical to these. You **must** run a new standard curve with each experiment.

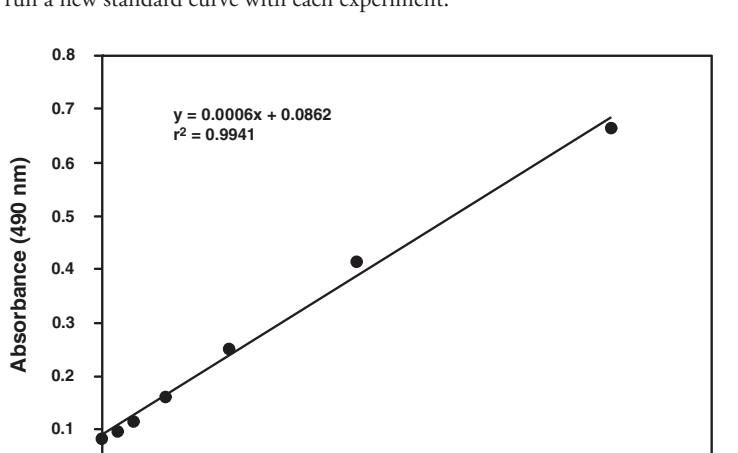


Figure 2. Typical standard curve
LDH standard was dissolved in 100 µl of RPMI 1640 without FBS. 100 µl of the reaction solution was then added to each well and incubated at room temperature for 30 minutes.

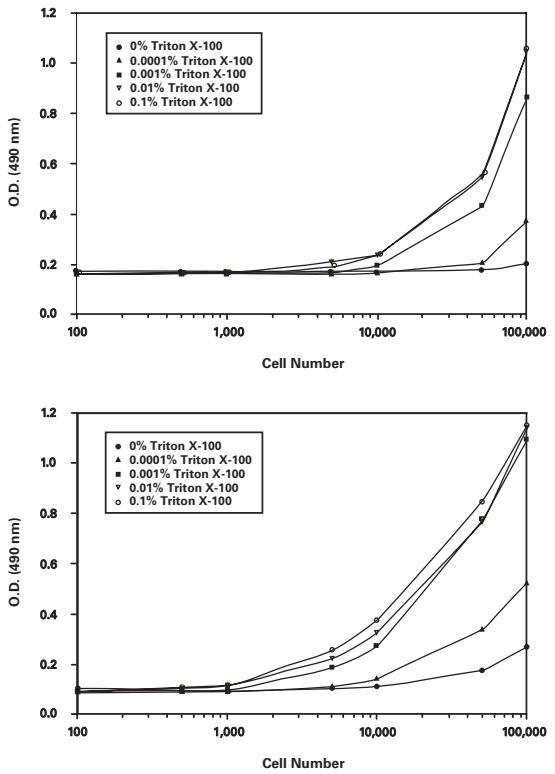


Figure 3. Release of cellular LDH by Triton X-100
Raw 264.7 cells (top panel, adherent cells) or Jurkat cells (bottom panel, suspension cells) were seeded in a 96-well plate in 100 µl of culture medium without FBS at the density indicated on the chart. The cells were incubated at 37°C in a CO_2 cell culture incubator. After several hours of incubation, the cells were treated with 0%, 0.0001%, 0.001%, 0.01%, and 0.1% of Triton X-100 and gently shaken for 10 minutes on an orbital shaker at room temperature. Samples were assayed according to the protocol described in **Performing the Assay** on page 9.

RESOURCES

References

1. Bonfoco, E., Krainc, D., Ankarcrona, M., *et al.* Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc. Natl. Acad. Sci. USA* **92**, 7162-7166 (1995).
2. Haslam, G., Wyatt, D., and Kitos, P.A. Estimating the number of viable animal cells in multi-well cultures based on their lactate dehydrogenase activities. *Cytotechnology* **32**, 63-75 (2000).
3. Wolterbeek, H.Th. and van deer Meer, J.G.M. Optimization, application, and interpretation of lactate dehydrogenase meaurements in microwell determination of cell number and toxicity. *ASSAY and Drug Development Technologies* **3(6)**, 675-682 (2005).

Related Products

Caspase-3 (human) Polyclonal Antibody - Cat. No. 160745
Caspase-8 (human recombinant) - Cat. No. 10007630
Caspase-9 Polyclonal Antibody - Cat. No. 160790
WST-1 Cell Proliferation Assay Kit - Cat. No. 10008883

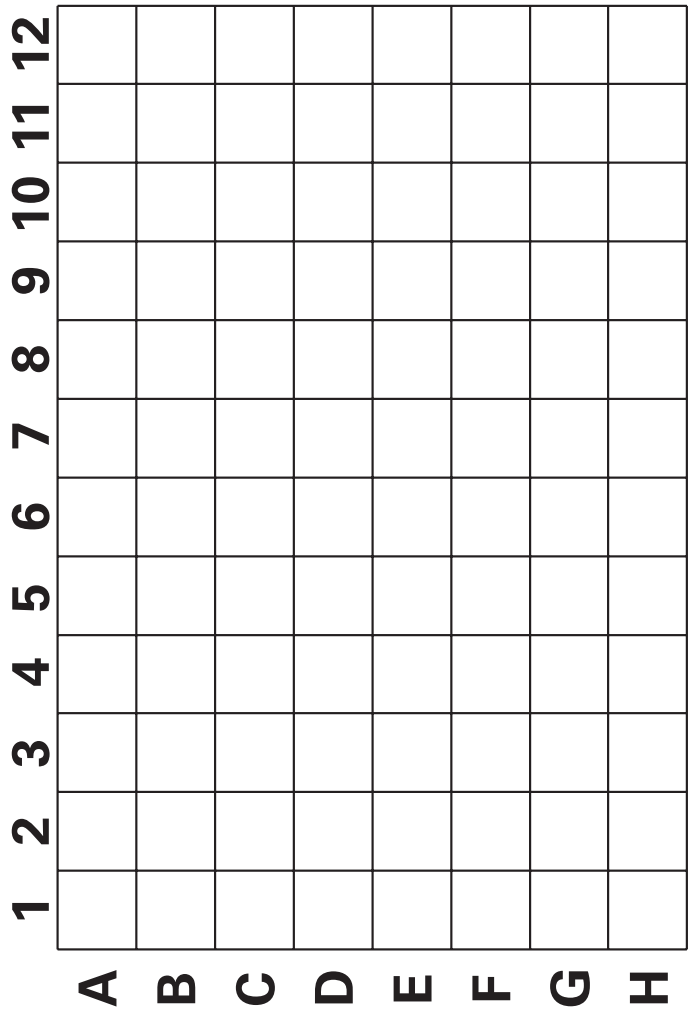
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



NOTES