LDH Cytotoxicity Assay Kit

LDH Cytotoxicity Assay Kit measures LDH activity in cell culture supernatant and it can be used to detect cytotoxicity for culture cells.

Catalog number: ARG81306

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Lactate dehydrogenase (LDH) is a wildly expressed soluble enzyme located in the cytoplasm of many different cell types. Upon cell death or cell membrane damage, LDH is released into the cell culture medium rapidly. Therefore, LDH activity in the medium can be used as a cytotoxicity marker.

PRINCIPLE OF THE ASSAY

This LDH Cytotoxicity Assay Kit provides a simple and reliable colorimetric method for measuring and monitoring cell cytotoxicity for both adherent and non-adherent cells. The kit contains sufficient reagents for 960 assays in 96-well plates. Cultured cells can be plated and then treated with compounds or agents to induce cytotoxicity.

Upon cell death, LDH is released into the cell culture media. The cell culture media is then transferred to a new plate (which is not provided in the kit) and the released LDH is then detected with cytotoxicity reagent.

The released LDH catalyzes the conversion the lactate which is included in the LDH Cytotoxicity Reagent to pyruvate and generates nicotinamide adenine dinucleotide (NADH). Then the WST-1 molecule, also present in the LDH Cytotoxicity Reagent, is converted from WST-1 to the orange formazan form with NADH. The intensity of the color is measured at a wavelength of 450nm. An increase in cell cytotoxicity is accompanied by increased LDH release and increased colorimetric signal.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
LDH Cytotoxicity Assay Reagent	10 ml (Ready-to-use)	-20°C (Protect from light)
10% Triton X-100 Solution	10 ml	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- 96-well clear cell culture plates

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The LDH Cytotoxicity Assay Reagent is a clear, slightly red, ready-to-use solution. Aliquot as needed to avoid repeated freeze-thaw cycles and store at -20°C protected from light. If precipitates or turbidity are observed upon thawing, warm the solution to 37°C for 5-10 minutes and agitate to dissolve the precipitates.
- It is highly recommended assaying the control and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Samples, negative and positive controls should be assayed in duplicates. Both negative and positive controls should be run alongside experimental samples.

1. Prepare a cell suspension containing 0.1-1.0 x 10^6 cells/mL in medium.
2. Add 150 µl of the suspended cells in the 96-well cell culture plate.
3. Keep 4 wells of cells as positive and negative controls. Add Cytotoxicity-mediating compound to be tested in the sample cells. Culture the cells for 24-96 hours at 37°C and 5% CO_2 in a humidified incubator.
4. Add 15 µl of sterile water into the wells contain samples and negative controls.
5. Add 15 µl of 10% Triton X-100 Solution into the positive wells.
6. Incubate the plate 5-10 minutes at room temperature.
7. Transfer 90 µl of medium from each well to a clean 96-well plate suitable for a plate reader.
8. Add 10 µl of LDH Cytotoxicity Assay Reagent into each well.
9. Incubate the plate at 37°C and 5% CO_2 for 0.5-4 hours.
10. Read the OD with a microplate reader at 450nm.

	Positive control	Negative control	Samples
Cell Suspension medium	150 µl	150 µl	150 µl
Compound to be tested	-	-	+
sterile water	-	15 µl	15 µl
Triton X-100	15 µl	-	-

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of controls and samples.
2. Positive control wells represent maximal LDH release, while negative control wells represent background of LDH release.
3. The OD for negative controls ($OD_{negative}$) is subtracted from both experimental samples ($OD_{samples}$)and positive control ($OD_{positive}$) OD values, and results are reported as a relative cytotoxicity percentage as below:

% Relative Cytotoxicity =

$$[(OD_{sample} - OD_{negative}) / (OD_{positive} - OD_{negative})] * 100\%$$

EXAMPLE OF RESULTS

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.