Lactate Dehydrogenase (LDH) Assay

Cat. #: CB001
Size: 2000 reactions

Kit components:

- Substrate Mix, lyophilizate, store at -20°C protected from light
- Assay Buffer, 0.6mL, store at -20°C protected from light.
  Prepare the 2X LDH assay buffer as follows: Dissolve 223 mg INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride), 57 mg PMS (N-methylphenazonium methyl sulfate) , 575 mg NAD (nicotinamide adenine dinucleotide), and 3.2 g lactic acid in 480 mL 200 mM Tris buffer solution, pH 8.0.
- 10X Lysis Buffer, 9% Triton X-100. Dissolve 9 mL Triton X-100 with 91 mL milli-Q water, 2.5mL, store at 4°C
- Stop Solution, 1 M acetic acid, 12mL, store at 4°C
- LDH Positive Control, 6µL, store at 4°C

Additional Materials Required

• Cultured cell line
• Tissue culture 96-well plate
• Flat-bottom, clear 96-well plate compatible with spectrophotometry
• 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS)
• Multichannel pipette
• Spectrophotometer (ELISA) plate reader capable of reading 490nm and 680nm absorbance

Description

The LDH Cytoxicity Detection is a colorimetric measure of cell cytotoxicity/cytolysis based on the measurement of LDH activity in the cell culture supernatant. LDH activity is determined via the

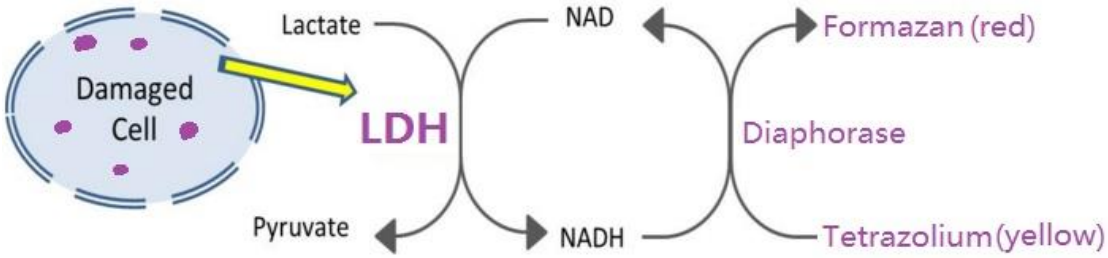

Fig.1 LDH detection mechanism

Diaphorase enzymatic reaction in which NAD+ is reduced to NADH/H+ by the LDH-catalyzed conversion of lactate to pyruvate, the H/H^+ from the resulting $NADH/H^+$ is transferred to the yellow tetrazolium salt INT, which becomes reduced to a red formazan product.

An increase in the number of dead or plasma membrane-damaged cells results in an increase in the total LDH enzymatic activity in the cell culture supernatant. This increase in LDH activity directly correlates with the amount of formazan product formed. In this assay, the value of spectral absorption of the resulting formazan at ~490nm is directly proportional to the number of damaged cells.

Reagent Preparation

Reaction Mixture

1. Dissolve one vial of the Substrate Mix (lyophilizate) with 11.4mL of ultrapure water in a 15mL conical tube. Mix gently to fully dissolve lyophilizate.

2. Thaw one vial of the Assay Buffer (0.6mL) to room temperature.
Note: Protect Assay Buffer from light and do not leave at room temperature longer than necessary.

3. Prepare Reaction Mix by combining 0.6mL of Assay Buffer with 11.4mL of Substrate Mix in a 15mL conical tube. Mix well by inverting gently and protect from light until use. Note: One vial of the Reaction Mixture is sufficient for two 96-well plates. The remaining Reaction Mixture can be stored at -20°C protected from light for 3-4 weeks with tolerance for three freeze/thaw cycles without affecting the activity within the storage period.

1X LDH Positive Control Dilute 1µL of LDH Positive Control with 10mL of 1% BSA in PBS.

Application

- Detection and quantification of cell-mediated cytotoxicity induced by cytotoxic T-lymphocytes (CTL), natural killer (NK) cells, lymphokine activated killer (LAK) cells or monocytes[12,13)]
- Determination of factors that lead to cytolysis[12)]
- Measurement of antibody-dependent cellular cytotoxicity (ADCC) and complement mediated cytolysis
- Determination of the cytotoxic potential of compounds in environmental and medical research and in the food, cosmetic, and pharmaceutical manufacturing[14-21)]
- Determination of cell death in bioreactors[22-24)]

Procedure

Cell Number optimization for LDH Cytotoxicity Assay

Different cell types have different levels of LDH activity. A preliminary experiment need be performed to determine the optimum number of cells to ensure LDH signal is within the linear range.

1. Take a serial dilution of cells (0-20,000 cells/100µL media) in two sets of triplicate wells in a 96-well tissue culture plate. A complete medium control without cells is used as blank and a serum-free media control without cells is used to determine the amount of LDH activity in sera.
2. Incubate the plate at 37°C, 5% CO_2 overnight.
3. Add 10µL of sterile, ultrapure water to one set of triplicate wells containing cells, these wells are referred to as Control LDH Activity
4. Add 10µL of Lysis Buffer (10X) to the other set of triplicate cell-containing wells and mix by gentle tapping. These wells are referred to as Total LDH Activity.
5. Incubate the plate in an incubator at 37°C, 5% CO2 for 45 minutes.
6. Take 50µL of each sample medium (e.g., serum-free medium, complete medium, Control LDH Activity, Total LDH Activity and 1X LDH Positive Control) to a 96-well flat bottom plate in triplicate wells.
7. Take 50µL of Reaction Mixture to each sample well and mix by gentle tapping.
8. Incubate the plate at room temperature for 30 minutes, avoid exposure to light.
9. Add 50µL of Stop Solution to each sample well and mix by gentle tapping.
10. Measure the absorbance at 490nm and 680nm. The LDH activity is determined by subtracting the 680nm absorbance value (background signal from instrument) from the 490nm absorbance.
11. Plot the Total LDH Release absorbance value minus the Control LDH Release absorbance value versus cell number to determine the linear range of the LDH cytotoxicity assay and the optimal number of cells.

Note: Do not create bubbles by pipetting; bubbles inhibit absorbance readings.

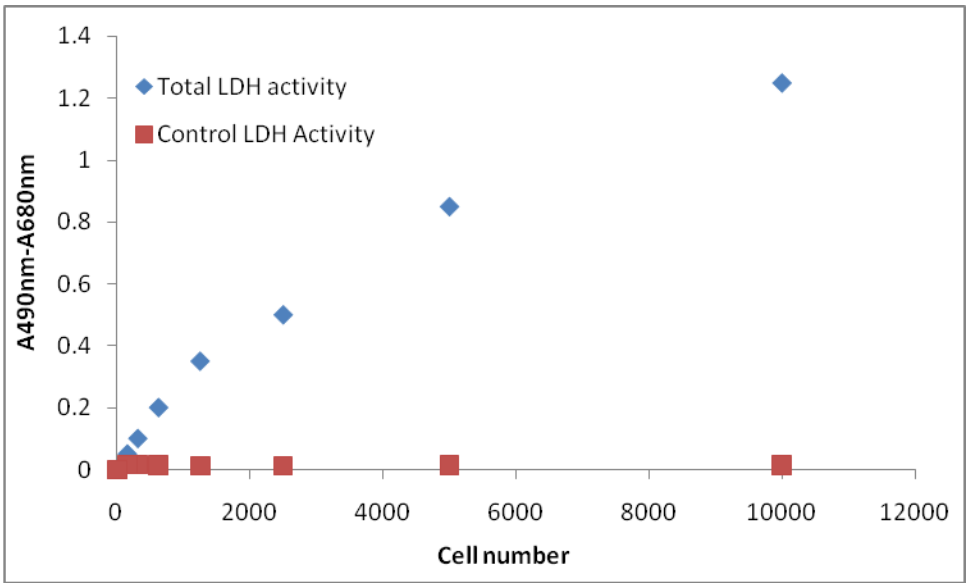

Figure 2. Graph of CHO-K1 cell LDH activity vs. cell number. CHO-K1 cells were diluted in a cell culture plate with RPMI 1640 medium containing 10% fetal bovine serum.

Activator/Inhibitor-Mediated Cytotoxicity Assay

1. Coat the optimal number of cells/well in 100µL of medium in triplicate wells in a 96-well tissue culture plate. Set wells for a complete medium control without cells is used for LDH background activity present in sera used for media supplementation and wells for a serum-free media control to determine the amount of LDH activity in sera. Plate additional cells in triplicate wells for Control LDH Activity (water) and Total LDH Activity (10X Lysis Buffer).
2. Incubate the plate at 37°C, 5% CO_2 overnight.
3. Prepare samples in the following manner:

• Control LDH Activity: Add 10µL of sterile, ultrapure water to one set of triplicate wells of cells.
• Total LDH Activity: Add nothing to one set of triplicate wells of cells.
• Activator/inhibitor treatment: Add 10µL of vehicle containing activator/inhibitor to one set of triplicate wells of cells.

4. Incubate the plate in at 37°C, 5% CO_2, as needed.
5. Add 10µL of Lysis Buffer (10X) to the set of triplicate wells serving as the Total LDH Activity, and mix by gentle tapping.
6. Incubate the plate in an incubator at 37°C, 5% CO2 for 45 minutes.
7. Take 50µL of each sample medium, including complete medium, serum-free medium, Control LDH Activity, activator/inhibitor-treated and Total LDH Activity) to a 96-well flat-bottom plate in triplicate.
8. Add 50µL of Reaction Mixture to each sample well and mix using a multichannel pipette.
9. Incubate the plate at room temperature for 30 minutes protected from light.
10. Add 50µL of Stop Solution to each sample well and mix by gentle tapping.
11. Measure the absorbance at 490nm and 680nm. LDH activity is determined by subtracting the 680nm absorbance value (background) from the 490nm absorbance before calculation of % Cytotoxicity [(LDH at 490nm) - (LDH at 680nm)].
12. To calculate % Cytotoxicity, subtract the LDH activity of the Control LDH Release (water-treated) from the Activator/inhibitor-treated sample LDH activity, divide by the total LDH activity [(Total LDH Release activity) –(Control LDH Release activity)], and multiply by 100:

$$\%\,\text{Cytotoxicity} = \frac{\text{Activator/inhibitor-treated activity} - \text{Control LDH activity}}{\text{Total LDH activity} - \text{Control LDH activity}} \times 100$$

Note: Do not create bubbles by pipetting; bubbles may inhibit absorbance readings.