Product Data Sheet

Product Name: Cytotoxicity LDH Assay Kit

Cat. No.: GK10003

Components

Components	100T	500T
Working Solution	5.5 mL	27.5 mL
Stop Solution	5.5 mL	27.5 mL
Lysis Solution	1.1 mL	5.5 mL

Features

Applications	Used to measure lactate dehydrogenase (LDH) activity in cell culture media. Used to determine the concentration of lactate dehydrogenase in a sample.
Shipping	Ship with blue ice.
Storage	Stored at -20°C protecting from light, and is stable for up to 2 years.
Usage	For Research Use Only! Not For Use in Humans.

Protocol

1. Control setup

High control: containing cells, culture medium, each sample adds 10 µL Lysis Solution, used to determine the maximum releasable quantity LDH of cells.
High blank control: containing culture medium, each sample adds 10 µL Lysis Solution, used to deduct the high control background absorbance value.
Low control: cells, culture medium, without lysis, used to measure spontaneous LDH release from untreated normal cells.
Background blank: containing culture medium only, used to deduct the background absorbance value of low control and sample wells.

	Sample	High Control	High Control Blank	Low Control	Background Blank
Culture medium	---	---	100µL	10µL	110µL
Cells	100µL	100µL	---	100µL	---
Treatment	10µL	---	---	---	---
Lysis Solution	---	10µL	10µL	---	---

Table 1. Control setup

Product Data Sheet

2. General Protocol

2.1 Pre-experiment (optimization of cell number)

(1) After washing the cells with the medium, prepare the cell suspension of 5×10^5 cells/ml.
(2) On a 96-well plate, add 100 µL of medium to each well.
(3) As shown in Figure 3, after adding 100 µL of cell suspension to row A of the 96-well plate (3 wells for high control and low control), Dilute the medium in 1/2 ratio with a multichannel pipette to form a serial cell gradient.
In addition, prepare 3 wells each for a high control blank (medium + Lysis Solution) and a background blank (medium only).
(4) Incubate the plate at 37°C in a CO_2 incubator.
* When incubate at 37°C in a CO_2 incubator for appropriate stage, ensure the incubation time setting is consistent with the cytotoxicity experiments.
(5) Add 10 µL of Lysis Solution to high control wells and high control blank wells, and add 10 µL of culture medium to low control wells and background blank wells.
(6) Incubate for another 30 min in a 37°C CO_2 incubator.
(7) Direct method: Remove 50 µL of the medium supernatant from each well, take the remaining medium and cells as the detection object, add 50 µL of Working Solution to each well, shake and mix well.
Indirect method: Pipette 50 µL of supernatant from each well into a new 96-well plate. Then add 50 µL of Working Solution to each well of this 96-well plate, shake and mix.
* To avoid aspirating the cells, aspirate the supernatant carefully.
* The direct method is suitable for other experiments that do not need to collect live cells, and the indirect method is suitable for other experiments that need to collect live cells. Choose one of the methods to measure LDH activity according to the purpose of the experiment.
(8) Add 50 µL of Working Solution to each well, protect from light by wrapping aluminum foil, etc., and incubate at room temperature.
* After adding Working Solution, the absorbance is proportional to the reaction time, it is recommended to detect within 0-30 min.
* Due to the great difference between different cells, it is recommended to measure the absorbance at 0 min, 5 min, 10 min, 20 min and 30 min respectively before the first experiment to determine the best reaction time.
(9) After adding 50 µL of Stop Solution to each well, immediately measure the absorbance at 490 nm with a microplate reader.
* Plot the absorbance as the X-axis and the cell concentration as the Y-axis, and select the best cell concentration according to the following requirements:
- The difference between the OD. values of the high control and the low control >0.2;
- The OD. value of this cell concentration on the linear curve is <2.0.

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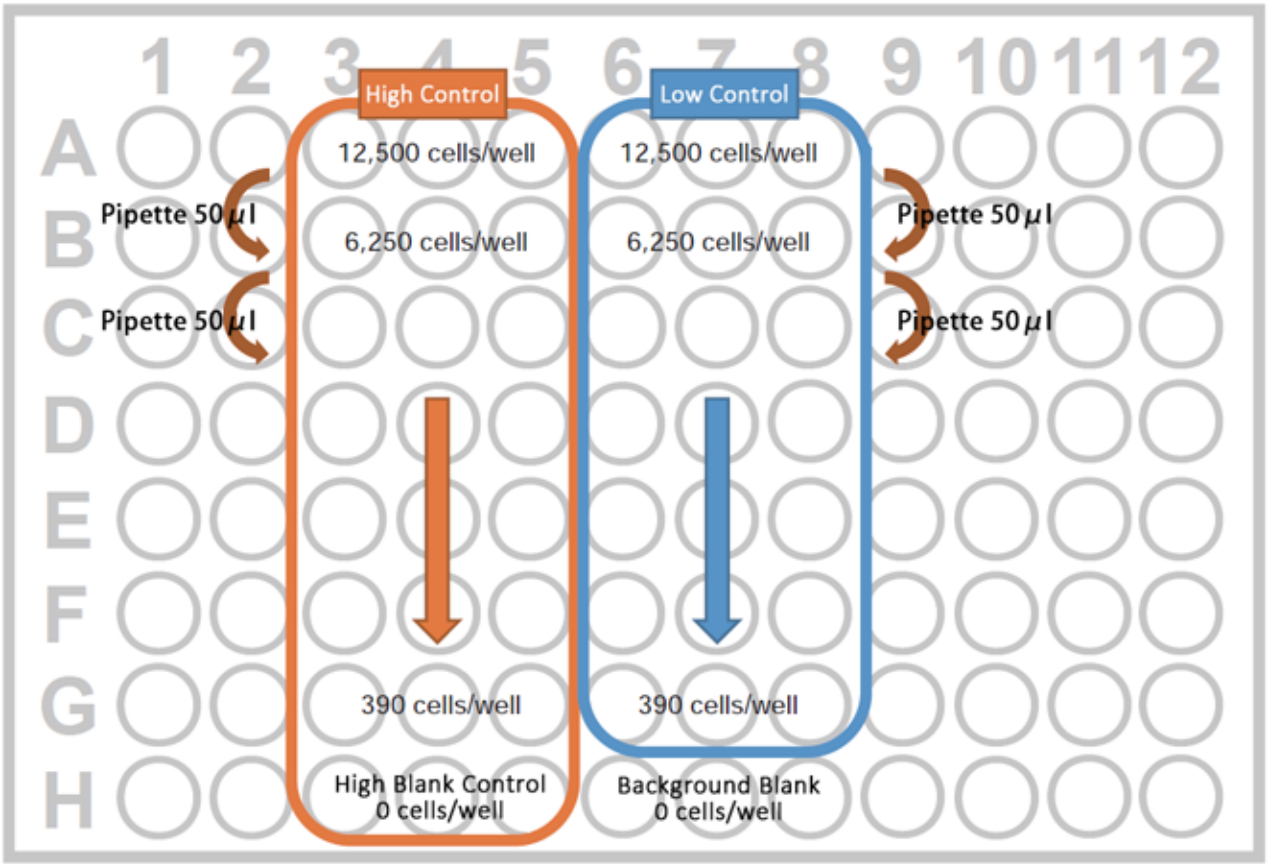

Fig 1. Example diagram of cell gradient configuration in pre-experiment on 96-well plate

2.2 Cytotoxicity assay

(1) Inoculate the cell suspension (100 µL/well) in a 96-well plate, and place the culture plate in an incubator for pre-culture for 24 hours.
(2) Add different concentrations of the treatment to be tested to the culture plate.
(3) Incubate the culture plate in the incubator for an appropriate time.
(4) Add 10 µL of Lysis Solution to the high control wells and high control blank wells, add 10 µL of medium to the low control wells, and incubate for 30 minutes in a 37°C CO_2 incubator.
(5) Direct method: Remove 50 µL of the medium supernatant from each well, take the remaining medium and cells as the detection object, add 50 µL of Working Solution to each well, shake and mix well.
Indirect method: Pipette 50 µL of supernatant from each well into a new 96-well plate. Then add 50µL of Working Solution to each well of this new 96-well plate, shake and mix.
* The direct method is suitable for other experiments that do not need to collect live cells, and the indirect method is suitable for other experiments that need to collect live cells. Choose one of the methods to measure LDH activity

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according to the purpose of the experiment.
(6) Incubate at room temperature in the dark.
* The incubation time is the same as that of the pre-experiment.
(7) After adding 50 µL of Stop Solution to each well, immediately measure the absorbance at 490 nm with a microplate reader.
* Use CCK-8 (GK10001) to obtain dead and live cell data.

2.3 Data processing

Cytotoxicity(%)=[(X-Z)/(Y-Z)] ×100%

X: Absorbance value of sample well - absorbance value of background blank well
Y: High control well absorbance value - high control blank well absorbance value
Z: Absorbance value of low control well - absorbance value of background blank well

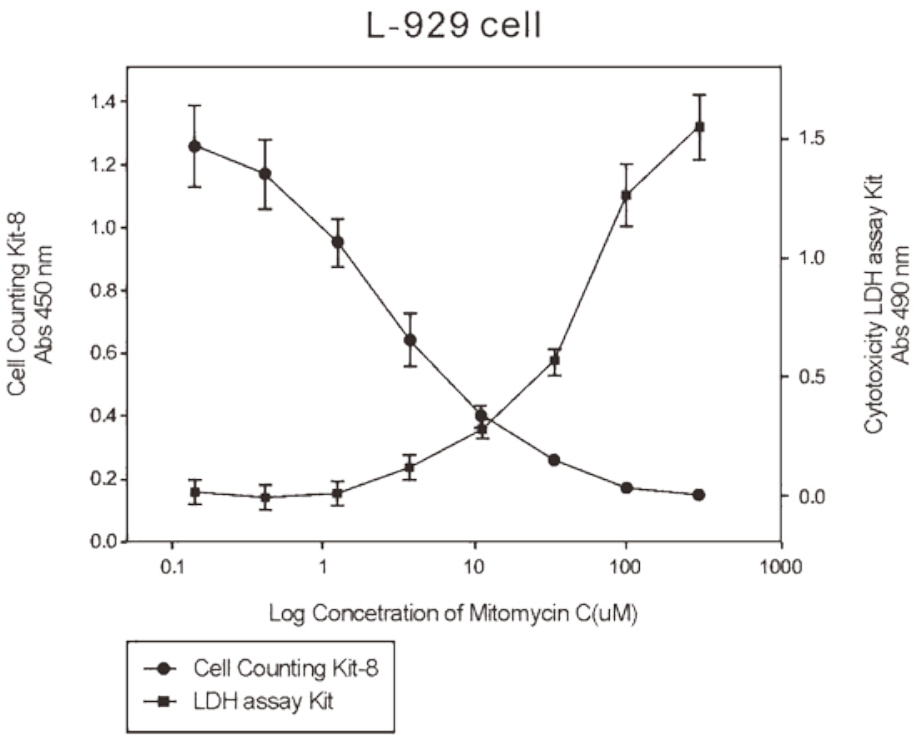

Fig 2. Analysis of cytotoxicity of mitomycin C on L-929 cells

3. Precautions

(1) Selection of 96-well plate: In the direct method, both adherent cells and suspension cells use a flat-bottom 96-well plate in the indirect method, a flat-bottom 96-well plate is used for adherent cells, and a round-bottom or V-

bottom 96-well plate is used for suspension cells.
(2) When using a 96-well plate for detection, if the cell culture time is long, be sure to pay attention to the evaporation problem. On the one hand, since the circle around the 96-well plate is the easiest to evaporate, you can discard the circle and add the same amount of PBS, water or culture medium; on the other hand, you can place the 96-well plate close to the incubator Place within the water source to ease evaporation.
(3) Make sure that there are no air bubbles in each well before testing with a microplate reader, otherwise it will interfere with the measurement.
(4) A multi-channel pipette is recommended to reduce the variation between parallel wells.
(5) Since serum contains lactate dehydrogenase, it is recommended that the concentration of serum should not exceed 1%, and it is best to use heat-inactivated serum. If 10% serum must be used, be sure to set a background blank (media only) during detection to eliminate background.
(6) Factors such as excessive cell growth, high density, high centrifugation speed, and large temperature difference between inside and outside the incubator will cause an increase of lactate dehydrogenase release from cells.

Background

The Cytotoxicity LDH Assay Kit is a kit for measuring cell damage by quantifying the activity of lactate dehydrogenase (LDH) released by cells into the culture medium. LDH is an enzyme present in the cytoplasm, and is released into the culture medium when the cell membrane is damaged. Since the released LDH is stable, measuring the amount of LDH in the medium can be used as an indicator to measure the quantity of dead and damaged cells.

Cytotoxicity LDH Assay Kit can measure LDH released by damaged cells. The principle is that LDH catalyzes lactic acid to generate pyruvate and NADH, NADH can reduce the water-soluble tetrazolium salt (yellow) to become formazan product (red) through the electron carriers, the absorbance of the formazan product is proportional to the concentration of LDH. Using this principle, the quantity of dead and damaged cells can be determined.

Fig1. The graphic illustration of Cytotoxicity LDH Assay Kit

The reagent in this kit will not react with alive cells, do not damage the cells. It can be directly added to the medium containing cells for detection (direct method). Cells can also be isolated and added to the culture medium for detection (indirect method, the isolated cells can be used for other experiments).

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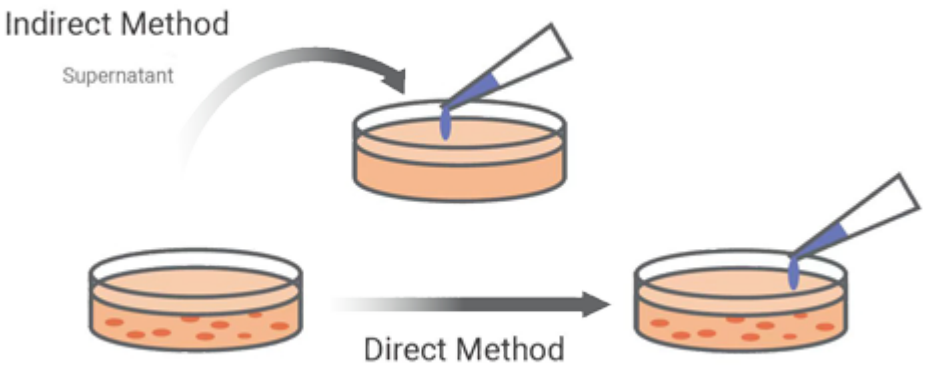

Fig2. Measurement of LDH activity by direct method or indirect method