NutriCulture LDH Cytotoxicity Assay Kit

100 tests

Cat No: LCA100

Shipping : Ship with wet ice.
Storage : Store at -20°C with protection from light.

Components

NutriCulture LCA Working Solution (5 ml)
NutriCulture LCA Stop Solution (5 ml)
NutriCulture LCA Lysis Solution (1 ml)

General Information

NutriCulture LDH Cytotoxicity Assay Kit is 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.

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.

NutriCulture LDH Cytotoxicity Assay 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).

Control setup

High control: Wells with cells, culture medium, and 10 µL Lysis Solution added during Step 4 of the protocol. This control is used to determine the maximum releasable quantity LDH of cells.

High blank control: Wells with culture medium and 10 µL Lysis Solution added during Step 4 of the protocol. This control is used to deduct the high control background absorbance value.

Low control: Wells with cells, culture medium, without Lysis Solution added. This control used to measure spontaneous LDH release from untreated normal cells.

Background blank: Wells with culture medium only. This control is used to deduct the background absorbance value of low control and sample wells.

Protocol

1. Seed 3000-10000 cells per well in 100 µl medium in a 96-well plate. Incubate cells at 37°C in a humidified CO2 incubator for 24 hours.

2. Apply various concentrations of substances to be tested to the cells.

3. Incubate the cells for an appropriate length of time (e.g. 12, 24, 48, 72 hours).

4. Add 10 µL of *NutriCulture* LCA 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. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.

5. Pipette 50 µL of supernatant from each well including controls and samples tested into a new 96-well plate.

6. Then add 50 µL of *NutriCulture* LCA Working Solution to each well of this new 96-well plate, shake and mix.

7. Incubate the plate at room temperature protected from light by wrapping aluminum foil.

> *After adding NutriCulture LCA 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 for each cell type.*

8. After adding 50 µL of *NutriCulture* LCA Stop Solution to each well, immediately measure the absorbance at 490 nm with a microplate reader.

> *The difference between the O.D. values of the high control and the low control is recommended to be >0.2;*
> *The O.D. value of the sample on the linear curve is recommended to be <2.0.*

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

For further information;
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