LDH Cytotoxicity Assay Kit

Cat #: KTA1030 Size: 96 T/480 T

	LDH Cytotoxicity Assay Kit		
REF	**Cat #:** KTA1030	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Storage: Stored at -20℃ for 6 months		

Assay Principle

Lactate dehydrogenase (LDH) is an oxidoreductase which catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. LDH is a stable enzyme, present in all cell types, and rapidly released into the cell culture medium upon damage of the plasma membrane. LDH, therefore, is the most widely used marker in cytotoxicity study. LDH Cytotoxicity Assay Kit provides a simple and easy colorimetric assay for the study of cytotoxicity. This assay is based on in a typical cytotoxicity assay, target cells are cultured with a cytotoxic chemical agent or a cytotoxic cell (NK cell, cytotoxic T cells) to induce target cell death and LDH release. The LDH-containing supernatants are transferred to wells of a new 96-well assay plate and mixed with the LDH Reaction solution. On the action of LDH, NADH reacts with the tetrazolium salt MTT to generate the reduced form of NAD^+ and MTT. The reduced form of MTT exhibits a maximum absorption at 565 nm. The intensity of the generated color correlates directly with the cell number lysed. After an incubation of 30 min at room temperature, the absorbance at 565 nm is read using a plate reader.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Assay Buffer	10 mL	50 mL	4℃
Lactic Acid Solution	5 mL	25 mL	4℃
MTT Solution	2 mL	10 mL	-20℃, protected from light
PES Solution	120 µL	600 µL	-20℃
LDH Positive Control	120 µL	300 µL	-20℃
NAD+ Solution	1 mL	5 mL	-20℃
Triton X-100 (10%)	10 mL	10 mL	4℃

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 565 nm
- Humidifying carbon dioxide incubator
- 96-well plate with clear flat bottom
- A plate centrifuge (Optional)
- Precision Pipettes, Disposable Pipette Tips
- Deionized Water

Reagent Preparation

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Lactic Acid Solution: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

MTT Solution: Ready to use as supplied. Keep on ice protected from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.

PES Solution: Ready to use as supplied. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.

LDH Positive Control: Ready to use as supplied. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.

NAD+ Solution: Ready to use as supplied. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.

Triton X-100 (10%): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

LDH Reaction Solution: To make 10 mL of LDH Reaction Solution, sufficient for use on one 96-well plate, by mixing 3.7 mL Assay Buffer, 1.4 mL MTT Solution, 800 µL NAD+ Solution, 100 µL PES Solution, 4.0 mL Lactic Acid Solution. Fresh reconstitution is recommended.

Assay Procedure

Ⅰ Determination of optimal target cell number

Different cell types contain different amounts of LDH. For cells with high LDH levels, fewer cells per well will be required to produce a strong A_{565} value than for cells with relatively low LDH levels. Therefore, we recommend performing an initial titration experiment to determine the optimal number of cells per well of the target cell you plan to use.

1. Seed target cells in a 96-well plate at a density of 10^3-10^6 cells/well in 200 µL of culture medium. Prepare six wells at each cell concentration.
2. Aspirate the growth medium from the cells. Wash the cells once with PBS, and the growth medium was replaced with a low serum medium containing 1% serum and incubated for an additional 1 h.
3. Add 200 µL of medium only (without cells) to three wells (background control), then add 20 µL of Assay Buffer.
4. Add 20 µL of Triton X-100 (10%) to three wells of each cell concentration (maximum release), and 20 µL of Assay Buffer to three wells of each cell concentration (spontaneous release), and incubate at room temperature for one hour.
5. Centrifuge the 96-well tissue culture plate at 400 g for 5 min (optional but recommended).
6. Transfer 100 µL of cell supernatant to a new 96-well assay plate.
7. Add 100 µL of LDH Reaction Solution to each well.
8. Incubate the plate for up to 30 min at 37℃.
9. Read the absorbance at 565 nm with a plate reader.
10. Assess the maximum release, spontaneous release and background, to determine the optimal number of cells per well of the target cell.

Ⅱ Sample Detection

1. Seed target cells in a 96-well plate at the previously determined optimal density in 200 µL of culture medium.

2. Aspirate the growth medium from the cells. Wash the cells once with PBS, and the growth medium was replaced with a low serum medium containing 1% serum and incubated for an additional one hour.
3. Add 200 µL of medium only (without cells) to three wells for background control, and to three wells for LDH Positive Control (optional).
4. Add 20 µL of 10% Triton X-100 solution to three wells containing cells (maximum release). Add 20 µL of Assay Buffer to three wells containing cells (spontaneous release) and to three wells medium only (background control). Add 20 µL of the LDH Positive Control to three wells medium only (optional).
5. Add 20 µL of experimental cytotoxic agent to appropriate wells in triplicate.
6. Incubate the plate in a CO_2 incubator at 37℃ for the length of time required by your experiment to induce cytotoxicity.
7. Centrifuge the 96-well tissue culture plate at 400 g for 5 min (optional but recommended).
8. Transfer 100 µL of cell supernatant to a new 96-well assay plate.
9. Add 100 µL LDH Reaction Solution to each well.
10. Incubate the plate for up to 30 min at 37℃.
11. Read the absorbance at 565 nm with a plate reader.
12. Subtract background A_{565} levels from all wells.

Note: The results of each experiment are calculated as "% cytotoxicity", or a percentage of the total amount of LDH contained within the target cells. Thus, for each experiment it is necessary to have a set of control wells in which all of the target cells are killed using 10% Triton X-100 solution provided in the kit. These are the "maximum release" well. Also, in each experiment it is necessary to have a set of control wells in which no cytotoxic agents or cytotoxic cells are added, resulting in only the lowest possible (spontaneous) LDH release. There are the "spontaneous release" wells. Cells treated with cytotoxic agents will release an amount of LDH that falls between the maximum release level and the spontaneous release level.

Data Analysis

That level will be calculated as a "% cytotoxicity" using the following formula:

Cytotoxicity of test sample (%)=$(A_{Sample}-A_{Spontaneous})/(A_{Maximum}-A_{Spontaneous})\times 100$

Where: A_{Sample}, the absorbance of Samples processed with cytotoxic agent at 565 nm, $A_{Spontaneous}$, the absorbance of sample spontaneous release at 565 nm, $A_{Maximum}$, the absorbance of sample maximum release at 565 nm.

Precaution

1. Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
4. Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
5. Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn.

Recommended Products

Catalog No.	Product Name
BMU106-EN	SuperKine™ Maximum Sensitivity Cell Counting Kit-8 (CCK-8)
KTD103-EN	Cell Proliferation Assay Cocktail
KTA2030	EdU Cell Proliferation Image Kit (Green Fluorescence)

KTA2031	EdU Cell Proliferation Image Kit (Orange Fluorescence)
KTA2010	TUNEL Apoptosis Detection Kit (Green Fluorescence)
KTA2011	TUNEL Apoptosis Detection Kit (Orange Fluorescence)
KTA2020	Cell Cycle Staining Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.