TECHNICAL MANUAL

Lactate Dehydrogenase (LDH) Cytotoxicity Colorimetric Assay Kit

- **SKU CODE:** MAES0196
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Cell culture and treatment
- LDH release measurement
- Data analysis and calculation

2. Intended use

This kit can be used to measure lactate dehydrogenase (LDH) release in cytotoxicity assays.

3. Detection principle

Lactate dehydrogenase catalyzes the reaction of lactic acid with NAD+ to produce pyruvic acid and NADH. NADH, under the action of PMS, transfers electrons to WST-8 to produce a yellow product, which has a characteristic absorption peak at 450 nm. Therefore, LDH activity can be quantified by measuring the OD value at 450 nm.

4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Lysis Solution	2 mL x 1 vial	10 mL x 1 vial	-20°C, 12 months
Reagent 2	Substrate	1.5 mL x 2 vials	15 mL x 1 vial	-20°C, 12 months, protect from light
Reagent 3	Chromogenic Agent	1.5 mL x 2 vials	15 mL x 1 vial	-20°C, 12 months, protect from light
Reagent 4	Stop Solution	1.5 mL x 2 vials	15 mL x 1 vial	-20°C, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Microplate centrifuge, Micropipettor, Water bath, Microplate reader (450 nm)

Reagents:

Double distilled water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use. Preheat stop solution at 37°C for 20 min in advance and use only after it is completely clear.
2. **Preparation of reaction working solution::** For each well, prepare 50 µL of reaction working solution (mix well 25 µL of substrate with 25 µL of chromogenic agent). The reaction working solution should be prepared fresh and stored protected from light.

7. The key points of the assay

1. According to the actual requirements, set different types of control wells.
2. The cells must be viable.
3. There should be no bubbles in the wells of the microplate when measuring the OD value.

8. Operating steps

The preparation of sample

1. 96-well cell culture plates are set up according to the following categories (each category with at least triplicate wells):

- Blank wells: 100 µL of culture medium with no cells (It is recommended to use low-serum medium containing 1% serum or serum-free medium)

- Sample control wells: 100 µL of cells for detection (with 5-10×10^3 cells)

- High control wells: 100 µL of cells for detection (with 5-10×10^3 cells)

- Sample wells: 100 µL of cells for detection (with 5-10×10^3 cells)

2. Incubate cells for 24 h in an incubator (5% CO_2, 100% humidity, 37°C).

3. Add 10 µL of culture medium into blank wells and sample control wells; Add 10 µL of drug stimulation with different concentrations into sample wells.

4. Incubate cells in an incubator (5% CO_2, 100% humidity, 37°C) (The incubation condition and time can be adjusted depending on the different cell type).

5. Take out 96-well cell culture plates from the cell incubator 1 h before the end of culture, add 10 µL of lysis solution into the high control wells, and mix repeatedly by pipetting.

6. Incubate cells for 1 h in an incubator (5% CO_2, 100% humidity, 37°C).

7. Centrifuge cells at 400×g for 5 min in the microplate centrifuge and collect the supernatant for detection.

Note: If there is no microplate centrifuge, the cells can be transferred to EP tubes and centrifuged using an ordinary centrifuge.

The measurement of sample

1. Prepare microplate and add 50 µL of supernatant into the corresponding blank, sample control, high control and sample wells.

2. Add 50 µL of reaction working solution to each well and mix thoroughly for 5 s using a microplate reader.

3. Incubate at 37°C for 10 min (The reaction time can be adjusted depending on color development. The plate can be read at multiple time points until the desired reading is observed. The OD value of high control should be < 2.0, while the OD value of sample control should be < 0.8).

4. Add 20 µL of stop solution to each well, mix and stop the reaction.

5. Measure the OD values of each well at 450 nm with microplate reader. The reference wavelength should be 600 nm, which when subtracted gives the required effective OD value.

9. Calculation

Cytotoxicity (%) = $(A_2 - A_1) \div (A_3 - A_1) \times 100\%$

[Note]

A_1: OD value of sample control well – OD value of blank well

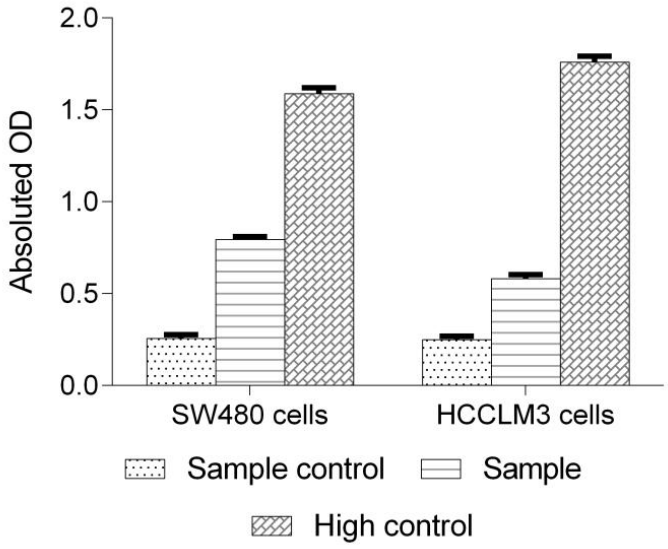
A_2: OD value of sample well – OD value of blank well

A_3: OD value of high control well – OD value of blank well

10. Appendix I Example Analysis

Example analysis:

Detection of SW480 cells and HCCLM3 cells (the concentration of protein is 4.40 g_{prot}/L) according to the protocol, the results are as follows:



11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.