TECHNICAL MANUAL

Lipid Peroxide Fluorometric Assay Kit

- **SKU CODE:** MAES0277
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can be used to measure lipid peroxide (LPO) in cell samples.

2. Detection principle

Lipid peroxides (LPO) are substances produced by the action of reactive oxygen species (ROS) on unsaturated fatty acids. The LPO produced by the reaction will continue to oxidize lipids, thereby accumulating LPO in a chain reaction. Excess LPO decomposes into highly active aldehydes such as acrolein, malondialdehyde (MDA), and 4-hydroxy-2-nonenal (4-NHE), causing cell toxicity and cell death.

This kit provides a fluorescent probe C11-BODIPY 581/591 that can detect LPO production. This fluorescent probe can react with the lipid free radicals in the lipid peroxidation pathway, enabling detection of lipid peroxidation levels in cells. The probe fluoresces red under normal conditions through photoexcitation, and the fluorescence changes from red to green with the progression of lipid peroxidation. Through the enhancement of green fluorescence intensity, LPO production can be detected with high sensitivity.

3. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer	50 mL x 2 vials	-20 °C, 12 months
Reagent 2	5 mmol/L Probe	0.05 mL x 1 vial	-20 °C, 12 months, protected from light
Reagent 3	100 mmol/L Positive Control	0.1 mL x 1 vial	-20 °C, 12 months, protected from light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

4. Materials prepared by users

Instruments:

Test tubes, Vortex Mixer, Centrifuge, Fluorescence Microplate reader, Fluorescence Microscope, Flow Cytometry

5. Reagent preparation

1. Equilibrate all reagents to room temperature before use. Aliquot 5 mmol/L probe and store at -20 °C. Avoid repeated freeze/thaw cycles.
2. **Preparation of buffer working solution::** Mix the buffer and double distilled water at a ratio of 1:9 thoroughly.
3. **Preparation of probe working solution::** Dilute 5 mmol/L probe with buffer working solution to the desired concentration. The recommended concentration is approximately 2-5 μmol/L, which can be adjusted according to experimental results. The working solution should be prepared fresh and protected from light, and should be used within 2 hours.
4. **Preparation of positive control working solution::** Dilute 100 mmol/L positive control with buffer working solution to the desired concentration. The recommended concentration is approximately 10-200 μmol/L, which can be adjusted according to experimental results. The working solution should be prepared fresh and protected from light, and should be used within 2 hours.

6. The key points of the assay

1. If using buffer working solution to wash and incubate cells, ensure a sufficient amount is prepared before the experiment.
2. The 5 mmol/L probe should avoid repeated freeze/thaw cycles. Before use, allow the 5 mmol/L probe to fully thaw and centrifuge until the liquid reaches the bottom of the tube before opening the cap. Prepare the fresh required amount of 5 mmol/L probe working solution before use.
3. The fluorescent substance is easily quenched, and it is best to measure within 2 hours after incubation to prevent fluorescence weakening.

7. Operating steps

Parameter setting of instrument

Green fluorescence

Fluorescence Microplate reader: Excitation: 500 nm; Emission: 540 nm

Flow Cytometry: FITC

Fluorescence Microscope: FITC or GFP

8. Procedure

1. Seed 2×10^5 cells into plate wells. The cells can be cultured and treated according to experimental needs.
2. Suspension cells: Transfer the cells into 2 mL EP tubes, centrifuge at 300×g for 5 min, then remove the medium. Wash the cells with buffer working solution 2-3 times. Adherent cells: Remove the medium and wash the cells with buffer working solution 2-3 times.
3. Set up different experimental groups: blank tube (normal cells only), control tube (normal cells only and loaded with probe), experiment tube (cells loaded with probe and treated with drug) and positive control tube (optional, cells loaded with probe and treated with positive control working solution).
4. Add buffer working solution to blank tube, add probe working solution to control, positive control and experiment tubes. Usually add 200 to 500 µL of probe working solution per 2×10^5 cells. Incubate the cells at 37 °C protected from light for 30-60 min. (The incubation time is related to cell type and fluorescence probe concentration, and the volume of liquid added should be consistent in all groups.)
5. Wash the cells with buffer working solution 2-3 times to remove probes that did not enter the cells.
6. Add buffer working solution to blank tube and control tube. Add appropriate drug to experiment tube. Add positive control working solution to positive control tube. In 2 mL EP tubes, usually add 200-500 µL of positive control working solution per 2×10^5 cells. The recommended concentration of positive control working solution is 10-200 µmol/L, and the stimulation time is 30-60 min. (The incubation time is related to cell type, drug concentration and positive stimulation concentration, and the volume of liquid added should be consistent in each group.)
7. Wash the cells with buffer working solution 2-3 times to remove excess drug and positive control working solution.
8. Detection: Add 100-200 µL buffer working solution to each tube of suspended cells to re-suspend cells, transfer to the detection carrier for detection. Adherent cells can be detected directly with a slide.

9. 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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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