Lipid Peroxidation Assay Kit (BODIPY 581/591 C11)

Introduction

Lipid peroxidation (LPO) is a core marker of biological membrane damage, and is widely involved in multiple pathological processes including atherosclerosis, neurodegenerative diseases, and ischemia-reperfusion injury. Importantly, lipid peroxidation serves as a key downstream signature of ferroptosis. During ferroptosis, iron-catalyzed chain-reaction oxidation occurs on polyunsaturated fatty acids within membrane phospholipids, leading to membrane disintegration and cellular dysfunction. Therefore, measurement of intracellular lipid peroxidation levels is critical for investigating disease mechanisms and ferroptosis regulation.

This kit utilizes the specific fluorescent probe BODIPY 581/591 C11 for rapid, high-sensitivity detection of lipid peroxidation in live cells. In its reduced state, BODIPY 581/591 C11 exhibits red fluorescence with excitation/emission at 581/591 nm. Upon oxidation of its polyunsaturated butadienyl moiety, its emission maximum shifts from 590 nm to 510 nm, producing green fluorescence with excitation/emission at 488/510 nm. Changes in the ratio of green-to-red fluorescence directly reflect lipid peroxidation levels. Compared with conventional methods, this probe is insensitive to environmental pH and polarity, features high photostability and low background noise, and is compatible with multiple detection platforms including fluorescence microscopes, flow cytometers and fluorescence microplate readers. It represents an ideal tool for studies on lipid peroxidation and ferroptosis.

Components and Storage

Components \ Size	100 Assays	500 Assays	Storage
BODIPY 581/591 C11 (1.5 mM)	100 μL	500 μL	-20°C away from light
LpoUp (1000X)	20 μL	100 μL	-20°C away from light
Shipping: Blue ice	**Shelf life: 1 year**		

Protocol

1. Working solution preparation: Dilute an appropriate volume of BODIPY 581/591 C11 (1.5 mM) 1:1000 in PBS to prepare BODIPY 581/591 C11 working solution at a final concentration of 1.5 μM. Prepare the working solution fresh and protect it from light.

***Note:**

a) The working concentration can be adjusted within the range of 1.5-10 μM according to experimental requirements.

b) Upon first use, aliquot BODIPY 581/591 C11 (1.5 mM) and store aliquots at -20°C or -80°C protected from light to avoid repeated freeze-thaw cycles. Storage at -80°C provides longer shelf-life.

2. Positive control setup: Add LpoUp (1000X) to cell culture medium at a 1:1000 dilution and treat cells for 4 h. For most cell types, LpoUp treatment induces massive accumulation of lipid peroxidation products. After BODIPY 581/591 C11 staining, treated cells exhibit markedly stronger green fluorescence relative to red fluorescence. By contrast, normal cells show predominant red fluorescence over green fluorescence after staining.

***Note:** The optimal dilution ratio and treatment duration of LpoUp (1000X) must be empirically determined for different cell lines. Certain cell types may show little or no response to LpoUp.

3. Cell detection

A. Adherent cells

1) Treat cells according to your experimental design. Remove culture medium and wash cells once with PBS.

***Note:**

a) For flow cytometry analysis, detach and harvest cells by trypsinization, resuspend, and follow the detection procedure for suspension cells.

b) For fluorescence microplate reader measurements, use black 96-well cell culture plates or black 96-well plates with transparent bottoms. Use top-reading mode for fully black plates and bottom-reading mode for transparent bottom plates.

2) Add sufficient BODIPY 581/591 C11 working solution to fully cover cells, and incubate at 37°C in the dark for 10-30 min. For 6-well plates, add 1 mL of BODIPY 581/591 C11 working solution per well.

***Note:** Incubation duration can be optimized for individual experiments; start with 10 min as the initial incubation condition.

3) After incubation, discard working solution and wash cells twice with PBS.

4) Add an appropriate volume of PBS and proceed to detection.

Reduced BODIPY 581/591 C11: Ex/Em = 581/591 nm

Oxidized BODIPY 581/591 C11: Ex/Em = 488/510 nm

B. Suspension cells

1) Treat cells according to experimental design. Count the cells, then centrifuge the cell suspension at 600 × *g* for 5 min at room temperature.

2) Discard supernatant. Resuspend cell pellet in BODIPY 581/591 C11 working solution to adjust cell density to 10^6-10^7 cells/mL. Incubate at 37°C in the dark for 10-30 min.

***Note:** Incubation duration can be optimized for individual experiments; start with 20 min as the initial incubation condition.

3) After incubation, centrifuge cells at 600 × *g* for 3-4 min at 4°C. Carefully remove supernatant without disturbing the cell pellet.

4) Resuspend cells in 1 mL PBS, and centrifuge at 600 × *g* for 3-4 min at 4°C. Discard supernatant, and repeat the PBS wash step once.

5) After the final wash, remove supernatant, resuspend cells in appropriate volume of PBS and perform detection.

Reduced BODIPY 581/591 C11: Ex/Em = 581/591 nm

Oxidized BODIPY 581/591 C11: Ex/Em = 488/510 nm

Note

1. Fluorescent dyes are susceptible to photobleaching. Protect reagents and samples from light during storage and handling to minimize quenching.
2. BSA and phenol red may interfere with assay results and should be avoided.
3. This kit is intended for live-cell detection only.
4. Aseptic conditions are required during operation; microbial contamination may compromise experimental results.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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