Staining Live Cells with Lipid Peroxidation Probe BDP® 581/591 C11

BDP® 581/591 C11 is a lipophilic ratiometric probe based on boron dipyrromethene, designed for monitoring oxidative stress in living cells. Thanks to its fatty acid tail (C11), the probe efficiently incorporates into cellular membranes, where it selectively reacts with lipid hydroperoxides and reactive oxygen species.

Oxidation of the dye's polyene chain shifts its fluorescence maximum from the red region of the spectrum (~594 nm) to the green region (~510 nm), enabling quantitative ratiometric analysis independent of probe concentration, excitation light intensity, and staining efficiency.

BDP® 581/591 C11 is suitable for long-term cell imaging and kinetic studies; it can be used to investigate ferroptosis and cell death, analyze oxidative stress induced by UV radiation, toxins, hypoxia, and other factors, screen for antioxidant compounds, and assess membrane integrity in metabolic studies.

Protocol

Solution Preparation

- **Stock Solution:** Dissolve the BDP®581/591 C11 probe in high-quality anhydrous DMSO to achieve a stock concentration ranging from 1 to 10 mM.

  Any remaining stock should be divided into aliquots and stored protected from light at -20 °C or -80 °C; avoid multiple freeze-thaw cycles.

- **Working Solution:** Immediately before use, dilute the stock to 1–10 µM (typically 2–5 µM) using an appropriate diluent, such as serum-free culture medium, HBSS, or PBS.

  The working concentration may be optimized for your specific cell type and experimental design; prepare a fresh solution each time.

Suspension Cell Staining

1. *For suspension cells:* Pellet the cells by centrifugation at 1000 × *g* for 3–5 minutes at 4 °C. Discard the supernatant and wash the cells twice with PBS, 5 minutes per wash.
2. *For adherent cells:* After two PBS washes, detach the cells with trypsin. Once fully detached, centrifuge at 1000 × *g* for 3–5 minutes.
3. Resuspend the cell pellet in 1 mL of the BDP®581/591 C11 working solution.
4. Incubate at 37 °C in the dark for 5–30 minutes. Optimal staining duration varies between cell types and should be determined empirically.
5. Following incubation, centrifuge at 1000 × *g* for 5 minutes, discard the supernatant, and wash the cells 2–3 times with PBS, 5 minutes each wash.
6. Resuspend the stained cells in pre-warmed serum-free medium or PBS for analysis by fluorescence microscopy or flow

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cytometry.

Adherent Cell Staining

1. Grow adherent cells directly on sterile coverslips placed in culture dishes.
2. Take the coverslip out of the medium, gently remove excess liquid, and place it in a humidified chamber to prevent drying.
3. Pipette 100 µL of the working solution gently onto one edge of the coverslip, then rock it slightly so that the dye spreads evenly and covers the entire cell surface.
4. Incubate at 37 °C in the dark for 5–30 minutes. The precise incubation time should be determined experimentally for each cell line.
5. After staining, remove the dye solution and rinse the coverslip 2–3 times with pre-warmed culture medium.

Cell Analysis

Evaluate the cells immediately using the following excitation/emission profiles for the reduced (non-oxidized) and oxidized states:

- **Reduced state:** Excitation 565–581 nm | Emission 585–591 nm (red channel)
- **Oxidized state:** Excitation 460–495 nm | Emission 510–550 nm (green channel)

Fluorescence Microscopy: Visualize using Texas Red (red channel) and FITC/GFP (green channel) filter cubes. Lipid peroxidation is quantified by the ratio of green-to-red fluorescence.

Flow Cytometry: Analyze using a 488 nm laser for excitation. Measure the green emission on the FITC channel (e.g., 530/30 nm) and the red emission on the PE/ECD channel (e.g., 585/42 nm or 610/20 nm) to calculate the shift.

Important! Fluorescent probes are susceptible to photobleaching. Minimize light exposure during all steps to preserve the signal.

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