Staining Live Cells with Endoplasmic Reticulum Stains LumiTracker® ER

LumiTracker® ER Green and LumiTracker® ER Red are cell-permeant, highly selective for the endoplasmic reticulum (ER) stains that can be used for live cell imaging. Both ER stains are derivatives of BDP dyes coupled with Glibenclamide (Glyburide). Glibenclamide binds to the sulphonylurea (SUR) receptors of ATP-sensitive potassium channels (K_{ATP}), which are prominent on the endoplasmic reticulum.

The staining is partially retained after fixation with formaldehyde. LumiTracker® ER Green and LumiTracker® ER Red are unsuitable for staining cells after fixation.

Note: The pharmacological activity of glibenclamide could potentially affect ER function. Variable expression of sulphonylurea receptors in some specialized cell types may result in non-ER labeling.

Preparation of solutions

1.1 Preparation of the stock solutions

- Dissolve 50 µg LumiTracker® ER Green in 64 µL DMSO to obtain 1 mM of stock solution.
- Dissolve 50 µg LumiTracker® ER Red in 55 µL DMSO to obtain 1 mM of stock solution.

Note: We recommend storing the stock solutions at -20 °C or -80 °C away from light. Avoid repeated freeze-thaw cycles.

1.2 Preparation of working solution

Dilute the stock solution of LumiTracker® ER stain in Hank's Balanced Salt Solution with calcium and magnesium (HBSS/Ca/Mg) to obtain the working solution. Use the concentration from 100 nM to 1 µM. The dilution of the LumiTracker® ER stain depends on the cell type and density and should be defined experimentally.

Cell staining

2.1 Suspension cell staining

1. Centrifuge cell suspension at 1000 *g* for 3-5 min; discard the supernatant.
2. Wash cells twice with prewarmed HBSS, 5 min each time at 37 °C.
3. The recommended cell density is 1×10^6 cells/mL.
4. Add 1 mL of prewarmed LumiTracker® ER working solution to the tube, and incubate cells for 15-30 min at 37 °C and 5% CO_2.
5. Centrifuge cells at 400 *g* for 3-4 min at room temperature; discard the supernatant.
6. Wash stained cells twice with medium.
7. Resuspend cells with serum-free cell culture medium or PBS.

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8. Analyze cells by flow cytometry.
9. For fluorescent microscopy, transfer a drop of the stained cell suspension onto a glass slide. Cover the cells with a coverslip.

2.2 Adherent cell staining

1. Grow cells on a sterile coverslip.
2. Adherent cells can be stained directly on the coverslip.
3. Aspirate the medium from coverslip.
4. Rinse cells with prewarmed HBSS at 37 °C.
5. Add 100 µL of prewarmed LumiTracker® ER working solution and gently shake it to cover the cells completely. Incubate cells for 15-30 min at 37 °C and 5% CO_2.
6. Wash stained cells twice with medium.
7. If detected by flow cytometry, cells need to be resuspended before staining.
8. For fluorescent microscopy, invert the coverslip with stained cells onto the slide to place them between the slide and the coverslip.

Cell fixation

1. If stained cells are to be fixed, use an incubation in 4% formaldehyde for 2 min at 4 °C.
2. Wash fixed cells twice in PBS for 5 min each time.
3. Mount cells under a coverslip using a mounting medium.

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