Mitotracking Dye

Mito-tracking Dye

Preparation of stock solutions

- Dissolve the lyophilized Mitotracker in DMSO to make the final concentration become 1mM. Store all other solutions of the Mitotracker dyes frozen at ≤-20°C and protected from light.

Cell Preparation, Staining, and Fixation

1. **Preparing staining solutions.** Dilute 1mM MitoTracker stock solution in appropriate buffer or growth medium. For staining cells that are to be fixed and permeabilized make the working concentration become 100-500nM.
2. **Staining adherent cells**. Growth cells on coverslips inside a petri dish filled with culture medium. Remove the media from the dish and add prewarmed (37 °C) staining solution containing MitoTracker probe. Incubate for 15-45 minutes under growth conditions. After staining is complete, replace the staining solution with fresh prewarmed media or buffer and observe the cells using a fluorescence microscope.
3. **Washing the cells.** Wash the cells in fresh and pre-warmed buffer or growth medium.
4. **Fixing the cells**. Remove the medium or buffer and replace it with freshly prepared, pre-warmed buffer or growth medium containing 2-4% formaldehyde. For MitoTracker Red CMXRos, 3.7% formaldehyde in growth medium works well.
5. **Rinsing the cells**. Rinse the cells several times in buffer.

DAPI

Preparation of DAPI Stock Solution

1. To make a 5mg/ml DAPI stock solution, dissolve one vial (10mg) in 2 ml of ddH_2O or dimethlformamide (DMF).
2. For long term storage, the stock solution can be aliquoted and stored at ≤-20°C. For short term storage, the stock solution can be kept at 2-6 °C and protected from light.

Counterstaining Adherent Cell

1. Equilibrate the sample with phosphate-buffered saline (PBS).
2. Dilute the DAPI stock solution to 300nM in PBS. Add ± 300µl of dilute DAPI staining solution to the coverslip. Make sure that the cells are completely covered.
3. Incubate for 1-5 minutes.
4. Rinse the samples with PBS several times. Drain excess buffer from the coverslip and mount using glycerol.
5. View the sample under confocal microscope with appropriate filters.