Mitochondrial Tracking Dye, Deep Red

Dyes and Stains

Fluorescent hydrophobic dye that selectively accumulates in mitochondria

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Catalog #100-0995

500 Tests

Product Description

Mitochondrial Tracking Dye, Deep Red is a fixable fluorescent dye that stains mitochondria in proliferating and non-proliferating cells, enabling quantification of mitochondrial activity, localization, and abundance. This hydrophobic dye easily crosses the membrane of live cells, and is partially dependent on mitochondrial membrane potential to accumulate in mitochondria. Upon depolarization, the dye relocalizes to the cytoplasm and becomes non-specific, but still retains its far-red fluorescence. Mitochondrial tracking dyes contain a cell-retaining group—thiol chloromethyl—that increases staining efficiency by retaining fluorescence long after fixation (Clutton et al.). Mitochondrial Tracking Dye, Deep Red is suitable for fluorescence microscopy and microplate assays.

Molecular Weight:	~700 g/mol
Excitation Wavelength:	640 nm
Emission Wavelength:	659 nm

Properties

Storage:	Store at -20°C.
Shelf life:	Product stable until expiry date (EXP) on label. Protect from prolonged exposure to light.
Format:	Red liquid

Directions for Use

Please read the entire protocol before proceeding. The following protocol is for staining cells in a black-wall/clear-bottom 96-well plate or on coverslips inside a Petri dish. If using other cultureware, adjust volumes accordingly.

Preparation of Mitochondrial Tracking Dye, Deep Red Working Solution

1. Thaw Mitochondrial Tracking Dye, Deep Red at room temperature (15 - 25°C).

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date as indicated on the label. Avoid repeated freeze-thaw cycles.
2. To prepare the Mitochondrial Tracking Dye, Deep Red working solution, add 20 µL of Mitochondrial Tracking Dye, Deep Red to 10 mL of Hanks' Balanced Salt Solution with 20 mM HEPES (HHBS) or a buffer of choice. Mix thoroughly. 10 mL of working solution is sufficient for one 96-well plate. Use the working solution immediately; do not store.

   NOTE: The optimal concentration of the working solution should be determined for different cell types.

Staining Samples

Refer to section A for staining adherent cells or section B for staining cells in suspension.

A. ADHERENT CELLS

1. Warm a sufficient volume of HHBS or a buffer of choice to 37°C.
2. Culture the cells in appropriate culture medium in either a black-wall/clear-bottom 96-well plate or on coverslips inside a Petri dish. When cells have reached the desired level of confluence, add an equal volume of Mitochondrial Tracking Dye, Deep Red working solution.
3. Incubate the cells at 37°C and 5% CO_2 for 30 minutes to 2 hours.

   NOTE: The optimal incubation time should be determined for different cell types.
4. Remove the Mitochondrial Tracking Dye, Deep Red working solution and wash cells with warm HHBS or a buffer of choice. Fill the wells or Petri dish with warm HHBS or culture medium.
5. If desired, fix the cells after staining using a suitable fixative. For nuclear counterstaining, DAPI (Hydrochloride) (Catalog #75004) may be used.

B. CELLS IN SUSPENSION

NOTE: Suspension cells may be attached to coverslips that have been treated with Corning® Cell-Tak™ Cell and Tissue Adhesive (Corning Catalog #354240), then stained as adherent cells.

1. Warm a sufficient volume of culture medium and HHBS or a buffer of choice to 37°C.
2. Centrifuge the cells at 300 - 500 x *g* for 5 minutes.

   NOTE: Centrifugation speed may need to be optimized to ensure cells are sufficiently pelleted.
3. Remove the supernatant and gently resuspend the cells in warm culture medium.
4. Add an equal volume of the Mitochondrial Tracking Dye, Deep Red working solution to the cells.
5. Incubate the cells at 37°C and 5% CO_2 for 30 minutes to 2 hours.

   NOTE: The optimal incubation time should be determined for different cell types.
6. Remove the Mitochondrial Tracking Dye, Deep Red working solution and wash cells with warm HHBS or a buffer of choice. Fill the wells with warm HHBS or culture medium.
7. If desired, fix the cells after staining using a suitable fixative. For nuclear counterstaining, DAPI (Hydrochloride) (Catalog #75004) may be used.

Fluorescence Detection

Observe stained cells using a fluorescence microscope equipped with appropriate filter sets.

NOTE: If the cells are not sufficiently stained, increase either the working solution concentration or the incubation time to allow the dye to accumulate in the cells.

References

Clutton G et al. (2019) A reproducible, objective method using MitoTracker® fluorescent dyes to assess mitochondrial mass in T cells by flow cytometry. Cytom Part A 95(4): 450–6.