PRODUCT INFORMATION

MitoLite™ Deep Red FX660

Item No. 25160

Ex./Em. Max: 640/659 nm
Supplied as: A solution in DMSO
Storage: -20°C
Stability: ≥2 years

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

MitoLite™ reagents are fluorogenic probes that can be used to label mitochondria within live cells. They are hydrophobic and easily cross live cell membranes to accumulate in mitochondria likely *via* the mitochondrial membrane potential gradient. MitoLite™ reagents contain a cell-retaining group, which allows them to remain in the mitochondria long-term, and their fluorescence can be measured using fluorescence microscopy, microplate fluorometry, or flow cytometry. MitoLite™ Deep Red FX660 displays excitation/emission maxima of 640/659 nm, respectively, and can be used for live and fixed cell applications.

Assay Protocol

1. **Prepare mitochondria-staining solution**
   a. Warm the MitoLite™ Deep Red FX660 solution to room temperature.
   b. Dilute 20 µl of 500X MitoLite™ Deep Red FX660 into 10 ml of Hanks balanced salt solution with 20 mM HEPES (HHBS) or buffer of your choice

Note 1: 20 µl of 500X MitoLite™ Deep Red FX660 yields a volume of mitochondria-staining solution sufficient for one 96-well plate. Aliquot and store unused MitoLite™ Deep Red FX660 stock solution at less than -15°C. MitoLite™ Deep Red FX660 is light sensitive. Light exposure and repeated freeze-thaw cycles should be avoided.

Note 2: The optimal working concentration is application specific. Staining conditions may be modified according to cell type and/or permeability.

2. **Prepare and stain the cells**
   a. Adherent cells:
      i. Grow cells in a 96-well black wall/clear bottom plate (100 µl/well) or on coverslips inside a petri dish filled with appropriate culture medium.
      ii. When cells reach the desired confluency, add an equal volume of the mitochondria-staining solution prepared in step 1b.
      iii. Incubate cells at 37°C, 5% CO_2 for 30 minutes to 2 hours.
      iv. Wash cells twice with pre-warmed (37°C) HHBS or buffer of your choice, then fill wells with buffer or growth medium.
      v. Observe cells using fluorescence technique of choice.
   b. Suspension cells*:
      i. Centrifuge the cells at 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.
      ii. Resuspend cell pellets in pre-warmed (37°C) growth medium and add an equal volume of mitochondria-staining solution prepared in step 1b to cells.
      iii. Incubate cells at 37°C, 5% CO_2 for 30 minutes to 2 hours.
      iv. Wash cells twice with pre-warmed (37°C) HHBS or buffer of your choice, then fill wells with buffer or growth medium.
      v. Observe cells using fluorescence technique of choice.

**Note 3: Suspension cells may be attached to coverslips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells.*

Note 4: If cells are not sufficiently stained it is recommended to increase either the labeling concentration or the incubation time to increase cellular dye accumulation.

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