PRODUCT INFORMATION

MitoLite™ NIR FX690

Item No. 25163

Ex./Em. Max: 660/692 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 or fixed 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™ NIR FX690 has been used to determine subcellular localization of photosensitizers modified by polyethylene glycol in HepG2 cells.[1] MitoLite™ NIR FX690 displays excitation/emission maxima of 660/692 nm, respectively, and can be used for live and fixed cell applications.

Assay Protocol

1. **Prepare mitochondria-staining solution**
   a. Warm the MitoLite™ NIR FX690 solution to room temperature.
   b. Dilute 20 µl of 500X MitoLite™ NIR FX690 into 10 ml of Hanks balanced salt solution plus 20 mM HEPES (HHBS) or buffer of your choice.

Note 1: 20 µl of 500X MitoLite™ NIR FX690 *yields a volume of mitochondria-staining solution sufficient for one 96-well plate. Aliquot and store unused* MitoLite™ NIR FX690 *stock solution at less than -15°C.* MitoLite™ NIR FX690 *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 100 µl 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.

Reference

1. Zou, Q., Zhao, H., Zhao, Y., *et al. J. Med. Chem.* **58(20)**, 7949-7958 (2015).

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