Mitochondrial Dyes

Product Description

Mitotracker dyes are fluorogenic stains for **staining mitochondria in live cells.** The dyes are membrane permeable and become brightly fluorescent upon accumulation in the mitochondrial membrane, without a wash step.

They are avalaible in blue, green, far-red and near-infra-red fluorescence.

Mitotracker 633 fluorescence is dependent on mitochondrial membrane potential, and the dye can be used to monitor changes in mitochondrial membrane potential in cells during apoptosis (Figure 6).

Mitotracker Green staining is not dependent on membrane potential, and can be used to stain fixed cells. Staining with Mitotracker 405 and Mitotracker 720 is partially dependent on mitochondrial membrane potential; with depletion of membrane potential, the localization of the dyes becomes non-specific, but fluorescence is not completely abolished. Mitotracker 650 staining is not dependent on mitochondrial membrane potential, but staining becomes dimmer and less specific after fixation.

Mitotracker Dyes

Product	Catalog no.	Size
Mitotracker 405	MT07007	20 x 50 ug
Mitotracker Green	MT45007	20 x 50 ug
Mitotracker 633	MT55007	20 x 50 ug
Mitotracker 650	MT57007	20 x 50 ug
Mitotracker 720	MT86007	20 x 50 ug

Storage and Handling

Store at -20°C and protect from light. Product is stable for at least 3 years from date of receipt when stored as recommended.

Spectral Properties

Component	Abs/Em	Detection channel	Membrane-potential dependent?
Mitotracker 405	398/440 nm	DAPI	Partial
Mitotracker Green	490/523 nm	GFP, FITC	No
Mitotracker 633	622/648 nm	Cy®5, APC	Yes
Mitotracker 650	644/670 nm	Cy®5, APC	No
Mitotracker 720	720/758 nm	Cy®5, Cy®7	Partial

See absorbance and emission spectra on page 3.

The optimal detection settings for Mitotracker 633 are the same as for Cy®5 and other far-red dyes. However, the dye also can be excited by the 555 nm laser, and has some fluorescence in the visible red channel.

While optimal for near-infrared imaging, Mitotracker 720 is bright enough to image in the far-red channel, and can be used for multi-color imaging with visible red probes.

Protocols

Reconstitution:
To prepare 200 uM stock solution, dissolve one 50 ug vial of lyophilized dye in anhydrous DMSO (or DMF as shown below. The stock solution can be stored in aliquots at -20°C desiccated and protected from light for at least 12 months.

Dye	Reconstitution volume
Mitotracker 405	475 uL
Mitotracker Green	400 uL
Mitotracker 633	460 uL
Mitotracker 650	625 uL
Mitotracker 720	441 uL

Live cell staining

1.When cells are at appropriate confluence, remove the medium and add pre- warmed medium containing 100 nM Mitotracker dye.
For suspension cells, pellet the cells and resuspend in medium containing diluted Mitotracker dye.

Note: The optimal staining concentration may vary by cell type and application. We recommend performing an initial test with the dyes at concentrations between 20-200 nM.
At higher concentrations, other structures may be stained.
Note: Alternatively, the dye can be added directly to the culture medium. We recommend making a dilute stock solution in culture medium to avoid exposing the cells to a transient high concentration of dye.
For example, dilute Mitotracker dye to 10 times the final desired concentration in culture medium, and then add 1/10 volume of the dilute stock to the medium on the cells and mix well by gently pipetting up and down.

2. Incubate cells for 15 minutes or longer at 37°C. Washing is not required before imaging.
Note: Longer staining times may result in brighter staining. Mitotracker dyes show no obvious toxicity at 100 nM in MCF-7 cells with incubation times up to 72 hours, but toxicity may vary by cell type.

3. Analyze fluorescence by fluorescence microscopy or flow cytometry using the appropriate excitation/ emission settings or detection channel (see Spectral Properties).

Note: Mitotacker dyes are not well-retained after fixation.
For fixed cell staining with Mitotracker Green, we recommend fixation before staining (see below). Other Mitotracker dyes cannot be use in fixed cells.

Staining of fixed cells (Mitotracker Green only)

1. Fix cells in 4% paraformaldehyde in PBS for 10 minutes at room temperature.
2. Following fixation, rinse cells in PBS and incubate with Mitotracker Green.
3. Rinse cells with PBS before imaging.

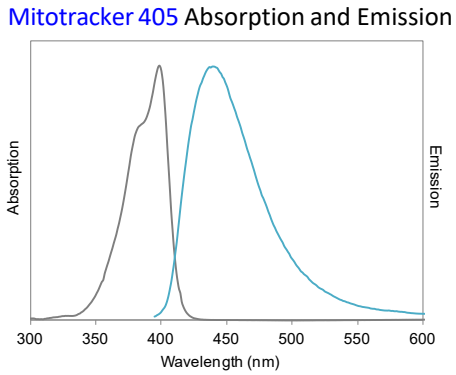

Figure 1. Normalized absorption and emission of Mitotracker 405.

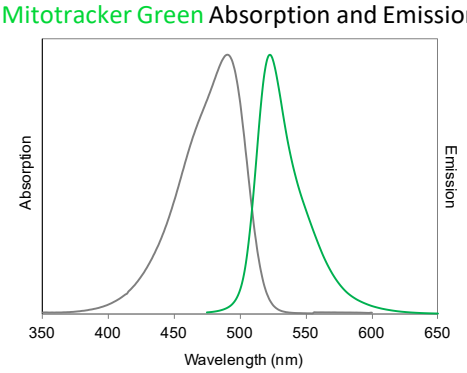

Figure 2. Normalized absorption and emission of Mitotracker Green.

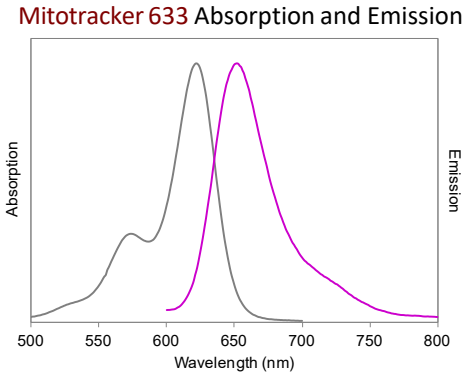

Figure 3. Normalized absorption and emission of Mitotracker 633.

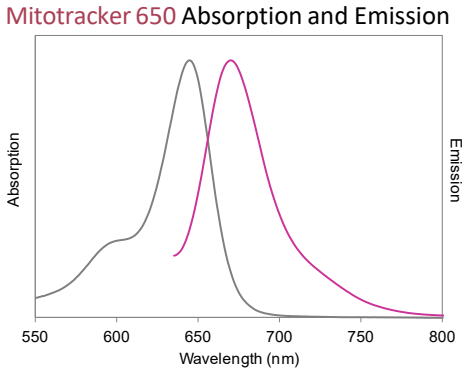

Figure 4. Normalized absorption and emission of Mitotracker 650.

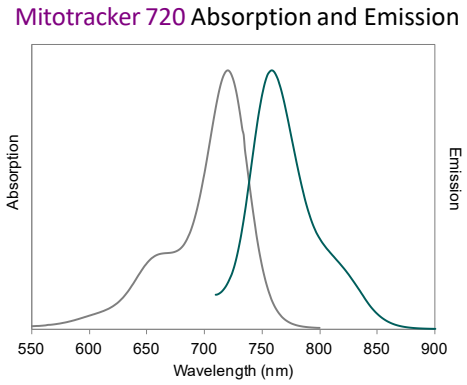

Figure 5. Normalized absorption and emission of Mitotracker 720.

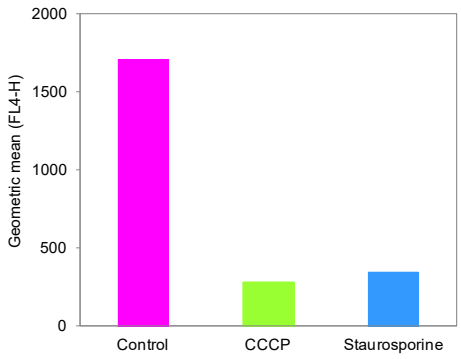

Figure 6. Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, then stained with Mitotracker 633.