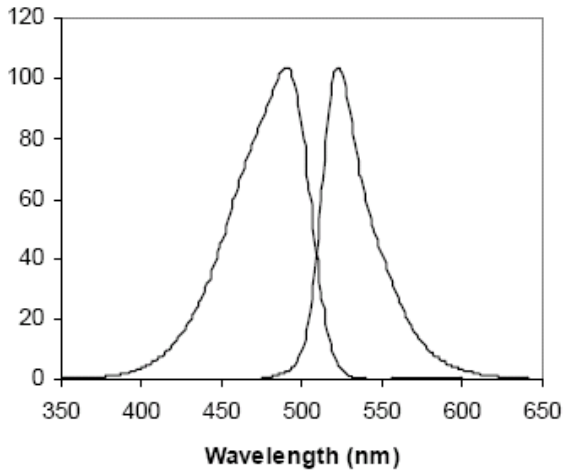
MitoGreen

Green-fluorescent mitochondrial dye

Instruction Manual

Catalog Number	PK-CA707-70054
Description	MitoGreen is a green fluorescent mitochondrial dye with properties similar to those of MitoTracker Green FM. The cell-permeant reagent is incubated at nanomolar concentrations, which diffuses across the plasma membrane and accumulates in the mitochondria. The dye is non-fluorescent until it partitions into the mitochondrial membrane. Mitochondria stained with MitoGreen become brightly fluorescent after accumulation of the dye in the lipid environment of mitochondria. The staining relies on mitochondrial mass, not on mitochondria membrane potential. Thus, the dye can be used to stain mitochondria in both live cells and or formaldehyde fixed cells. Live cells stained with the dye may be fixed with formaldehyde. MitoGreen is spectrally similar to FITC, making it optimally excitable by the 488 nm argon laser line. PromoKine also offers MitoRed, a novel far-red mitochondrial membrane potential dye, as well as a selection of classic mitochondrial membrane potential dyes.
Quantity	20 x 50 µg
Appearance	Red-orange powder
Absorption/Emission Spectra	Ex/Em maxima: 490/523 nm  Absorption and emission spectra of MitoGreen in methanol.

Applications	To prepare a 200 µM stock solution, dissolve a 50 µg tube of lyophilized product in 400 µL anhydrous DMSO or DMF. The concentration of MitoGreen for optimal staining will vary by application and cell type. The staining protocols provided here are general guidelines and may need to be optimized. Dilute the MitoGreen stock solution to the final working concentration in cell culture medium. For live cell staining, working concentrations of 20-200 nM are recommended. At higher concentrations, this probe may stain other cellular structures. Live cells stained with MitoGreen can be fixed but fluorescence is not well-retained. Subsequent permeabilization steps may also affect staining. *Procedure* <u>Staining of adherent cells:</u> 1. Grow cells on coverslips in a dish or directly onto dish if slide mounting is not desired. 2. When cells are at appropriate confluency, remove the medium and add prewarmed medium containing diluted MitoGreen. Alternatively, the probe can be added directly to the current culture medium. 3. Incubate cells for 30 minutes (or longer). 4. Replace the loading solution with fresh medium or PBS and image cells using a fluorescence microscope. <u>Staining of suspension cells</u>: 1. Pellet cells and aspirate the supernatant. 2. Resuspend pellet in medium containing diluted MitoGreen. 3. Incubate for 30 minutes (or longer). 4. Centrifuge the cells and resuspend pellet in fresh medium or PBS and image cells using a fluorescence microscope. Note: If adherent or suspension cells are not stained sufficiently, increase the concentration or the incubation time for the dye to accumulate in the mitochondria. <u>Staining of fixed cells:</u> 1. MitoGreen may be used to stain cells fixed in formaldehyde. We recommend 3.7% formaldehyde in PBS for 10 minutes as a fixative. 2. Following fixation, rinse cells in PBS and incubate with MitoGreen. Rinse cells at least once with PBS before viewing. [Note: The concentration of the probe and staining time may differ between fixed and live cells.] Note: Live cells stained with MitoGreen can be fixed but fluorescence is not well-retained. Subsequent permeabilization steps may also affect staining.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage	MitoGreen stock solutions can be stored in frozen aliquots at -20°C for at least 6 months and should be protected from light.

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