Revised: January 6, 2021

Product Information

MitoView™ Dyes

Product	Ex/Em (nm)	Membrane-potential dependent	Catalog no.	Size
MitoView™ 405	398/440	Partial	70070-T	50 ug
			70070	20 x 50 ug
MitoView™ Green	490/523	No	70054-T	50 ug
			70054	20 x 50 ug
MitoView™ 633	622/648*	Yes	70055-T	50 ug
			70055	20 x 50 ug
MitoView™ 650	644/670	No	70075-T	50 ug
			70075	20 x 50 ug
MitoView™ 720	720/758**	Partial	70068-T	50 ug
			70068	20 x 50 ug

See absorbance and emission spectra on page 2.

*The optimal detection settings for MitoView™ 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, MitoView™ 720 is bright enough to image in the far-red channel, and can be used for multi-color imaging with visible red probes.

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.

Product Description

MitoView™ dyes are fluorogenic stains for staining mitochondria in live cells. The dyes accumulate within the cell and preferentially localize in the mitochondrial matrix, induced by the greater negative membrane potential of mitochondria in live cells compared to the plasma membrane potential.

MitoView™ 633 is potential-dependant and accumulates in mitochondria in proportion to the electron gradient. The dye can be used to monitor changes in mitochondrial membrane potential in cells during apoptosis (Figure 1).

MitoView™ Green is a potential-independent dye that accumulates in the mitochondria. The fluorescence of cells stained with this dye is directly proportional to the mitochondrial content and has been validated to quantify mitochondrial mass using flow cytometry (1, 2). MitoView™ Green can be used to stain mitochondria in live as well as formaldehyde-fixed cells, but fixed cell staining tends to be less specific compared to live cell staining. MitoView™ Green staining is also not compatible with solvent-based fixatives or permeabilization. For a general protocol for staining fixed cells see page 2.

Staining with MitoView™ 405 and MitoView™ 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. MitoView™ 650 staining is not dependent on mitochondrial membrane potential, but staining becomes dimmer and less specific after fixation.

For fixed cell staining, we recommend using one our CF™ Dye conjugated mitochondrial marker antibodies. Biotium also offers a selection of classic dyes useful for measuring mitochondrial membrane potential, including JC-1, TMRM, TMRE, Nonyl Acridine Orange (NAO), and Rhodamine 123 (see related products).

References

1) Science Adv. 6, 46(2020); 2) Cell Mol Immunol (2020)

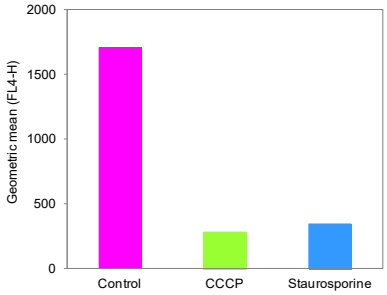

Figure 1. Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, then stained with MitoView™ 633.

Protocols

Reconstitution

To prepare 200 uM stock solution, dissolve one 50 ug vial of lyophilized dye in anhydrous DMSO (catalog number 90082) 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
MitoView™ 405	475 uL
MitoView™ Green	400 uL
MitoView™ 633	460 uL
MitoView™ 650	625 uL
MitoView™ 720	441 uL

Live cell staining

1. When cells are at appropriate confluence, remove the medium and add pre-warmed medium containing 100 nM MitoView™ dye. For suspension cells, pellet the cells and resuspend in medium containing diluted MitoView™ 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 an intermediate dilution of dye in culture medium to avoid exposing the cells to a transient high concentration of dye. For example, dilute MitoView™ dye to 10 times the final desired concentration in culture medium, and then add 1/10 volume of the intermediate dilution 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. MitoView™ 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.

   Note: MitoView™ dyes are not well-retained after fixation. For fixed cell staining with MitoView™ Green, we recommend fixation before staining (see page 2). Other MitoView™ dyes cannot be used in fixed cells.

Staining of fixed cells (MitoView™ 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 MitoView™ Green for 15 minutes or longer at room temperature.
3. Rinse cells with PBS before imaging.

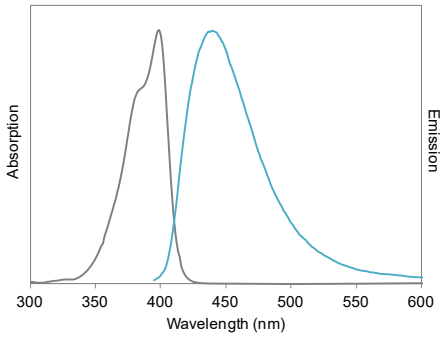

Figure 2. Normalized absorption and emission of MitoView™ 405.

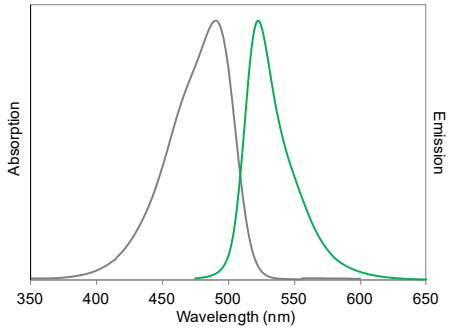

Figure 3. Normalized absorption and emission of MitoView™ Green.

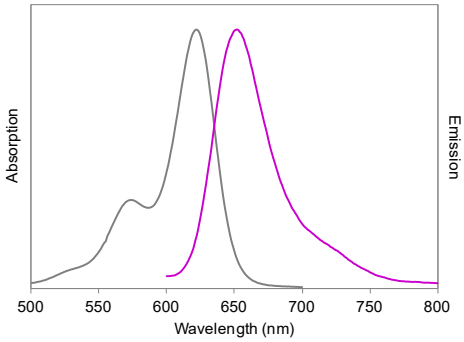

Figure 4. Normalized absorption and emission of MitoView™ 633.

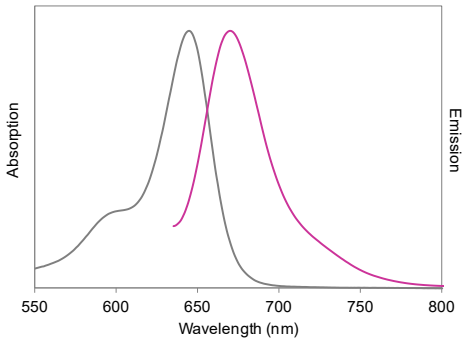

Figure 5. Normalized absorption and emission of MitoView™ 650.

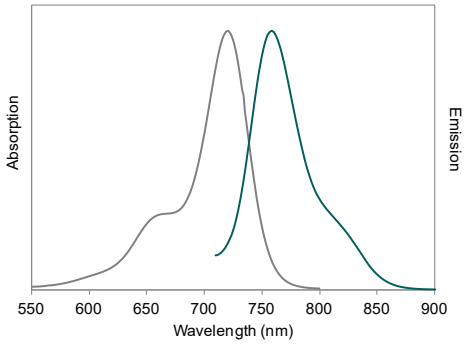

Figure 6. Normalized absorption and emission of MitoView™ 720.

Related Products

Catalog number	Product
90082	DMSO, anhydrous
22023	Paraformaldehyde, 4% in PBS Ready-to-Use Fixative
30001	JC-1 Mitochondrial Membrane Detection Kit
70016	TMRE
70017	TMRM
70010	Rhodamine 123
70012	Nonyl Acridine Orange (NAO)
30062	NucView® 488 and MitoView™ 633 Apoptosis Assay Kit
70066	LysoView™ 405
70067	LysoView™ 488
70061	LysoView™ 540
70058	LysoView™ 633
70059	LysoView™ 650
70065	LipidSpot™ 488 Lipid Droplet Stain
70069	LipidSpot™ 610 Lipid Droplet Stain
70064	ViaFluor® 405 Live Cell Microtubule Stain
70062	ViaFluor® 488 Live Cell Microtubule Stain
70063	ViaFluor® 647 Live Cell Microtubule Stain
40081	NucSpot® Live 488 Nuclear Stain
40082	NucSpot® Live 650 Nuclear Stain
40060	RedDot™1 far-red nuclear stain for live cells
40061	RedDot™2 far-red nuclear stain for dead or fixed cells
30090	CellBrite™ Fix 488 Membrane Stain
30088	CellBrite™ Fix 555 Membrane Stain
30089	CellBrite™ Fix 640 Membrane Stain
30105	CellBrite™ Steady 405 Membrane Staining Kit
30106	CellBrite™ Steady 488 Membrane Staining Kit
30107	CellBrite™ Steady 550 Membrane Staining Kit
30108	CellBrite™ Steady 650 Membrane Staining Kit
30109	CellBrite™ Steady 685 Membrane Staining Kit

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® dye conjugates of transferrin, cholera toxin, dextrans, lectins, and annexin V for cellular imaging, plus many more fluorescent probes and kits for cellular and molecular biology research.