Nuclear Labels

Dye	Ex_{max}	Em_{max}	Concentration	LM Filter	Laser Line	MRC-1024 Filter
Syto 24/21	490/494	515/517	2 μM – 6μM	FITC	488 nm	522DF35
Yo-Pro-1	491	509	2 μM – 6μM	FITC	488 nm	522DF35
Lo-Pro-1	567	580	2 μM – 6μM	Cy3	568 nm	605DF32
To-Pro-3	642	661	2 μM – 6μM	Cy5	647 nm	680DF32

Dyes are diluted in Embryo Medium from 1 mM stocks in DMSO. The stocks are kept frozen at -20° C. They may be aliquotted into small vials to avoid repeated freeze-thaw cycles and the attendant risks of water absorbtion and degradation. Neuromast sensory cells will stain within 20 minutes, although 1 hour seems better for timelapse imaging. The fluorescence of these dyes increases upon binding to DNA such that it is possible to use them without washing. All labels persist for at least 48 hours following incubation in live cells. Each of these nuclear labels are retained after fixation by 2% and 4% paraformaldehyde.

The monomeric cyanine dyes are cell impermeant in most cell types, which explains their apparent specificity for sensory cells. Their entry into live neuromast sensory cells is mediated by channels of unknown identity. The monomeric cyanine dyes display no increase in the number of cell types labeled by up to 2 hours of dye exposure. They can be employed as DNA counterstains in fixed samples. To-Pro-3 is particularly dependent upon concentrations over 2 μM to resist bleaching. Lo-Pro is of limited utility due to a relatively broad spectra that fluoresces into the green channel upon excitation by blue wavelengths.

Syto dyes are permeant DNA labels that label nuclei only in live cells. Hair cells are strongly labeled and support cells are weakly labeled with Syto 24 at 30 min. Prolonged exposure to Syto 24, up to 2 hours, increases support cell nuclear intensity and labels additional cells in the epithelium. Syto dyes will not label DNA after aldehyde fixation. Syto dyes 21 and 24 were chosen over the other green Syto variants for desirable spectral properties and that they do not exhibit detectable RNA labeling.

Syto dyes are believed to interfere with transcription. Their effect on viability and development in long term live cell studies must be assessed for any experiment. Molecular Probes recommends that Syto dyes are only used in plastic containers to avoid non-specific binding to glass, and that phosphate-containing buffers be avoided for preparing working solutions.

Mitochondrial Labels

Dye	Ex_{max}	Em_{max}	Concentration	LM Filter	Laser Line	MRC-1024 Filter
DASPEI	461	589	5 drops/2 ml	Green LP or FM1-43	488 nm	522DF35+ 605DF32
Mitofluor Green	490	516	25 nM	FITC	488 nm	522DF35
Mitotracker Green FM	490	516	25 nM	FITC	488 nm	522DF35
Mitotracker Orange	554	576	50 nM	Cy3	568 nm	605DF32
Mitotracker Red	579	599	50 nM	Cy3/TR	568 nm	605DF32

DASPEI is prepared as a stock solution, 40 mg/100 ml distilled water, stored at 4° C. The Mitotracker and Mitofluor dyes are received as 50 µg vials to which anhydrous DMSO is added to produce a 1 mM stock. These stocks are kept frozen at -20°C. Small aliquots are recommended to avoid repeated freeze-thaw cycles. The reduced forms are particularly prone to oxidation. Working solutions of these dyes are dissolved in Embryo Medium. Label will appear in 15 minutes incubation, but they are commonly incubated for 1 hour when preparing fish for timelapse imaging.

DASPEI primarily labels hair cell mitochondria but also labels the nerve to the neuromast. The DASPEI emission curve is very broad, appearing in both "green" and "red" channels, giving yellow mitochondria and weakly red nuclei. Unlike the Mitotrackers, DASPEI will not label support cells and only labels a sub-population of hair cells in the neuromast. This label will leave the mitochondria after intense imaging on the confocal, presumably due to some form of phototoxic response causing mitochondrial depolarization.

The red and orange Mitotrackers will only label mitochondria possessing an electropotential difference relative to the cytoplasm. Mitotracker Green FM appears to accumulate regardless of mitochondrial membrane potential, making it a good marker for total mitochondrial mass. All Mitotrackers possess a weakly reactive chloromethyl group that forms thiol-peptide bonds with mitochondrial proteins. This allows them to be retained after fixation, however, they are also retained if the mitochondria become depolarized.

Mitofluors label mitochondria in a manner similar to the corresponding Mitotrackers. Howerver, they are not retained by paraformaldehyde fixation. The Mitofluors are also retained upon depolarization of the mitochondria.

Higher concentrations of Mitotrackers and Mitofluor lead to dramatically increased non-specific background staining in surrounding epithelium as well as stain mitochondria in support cells and epithelial cells. The epithelial cells display a distinct mitochondrial

pattern that allows ready distinction from neuromast cells. The reduced versions, Red CM-H_2-XRos and Orange CM-H_2-TMRos, are preferred since they are non-fluorescent until entering an actively respiring mitochondrion and oxidized. Mitotracker Green FM is non-fluorescent until entering the lipid environment of the mitochondrial wall.

Transduction Channel Label

Dye	Ex_{max}	Em_{max}	Concentration	LM Filter	Laser Line	MRC-1024 Filter
FM1-43	510	626	3 µM	FM1-43*	488 nm	605DF32
FM1-43FX	510	626	3 µM	Cy3	488 nm	605DF32

*A Cy3 or Rhodamine filter will work. The Marianas FM1-43 filter uses a 490/20 excitation filter and the 617/73 emission filter. The Axioplan FM1-43 filter cube contains a 480/30 exciter and a 570 long pass filter.

FM1-43 is received as 100 µg dry powder reconstituted to 817 µM with 200 µl DMSO. It is dissolved in Embryo Medium for labeling zebrafish, with an incubation time of 30 to 45 seconds. Longer exposure to FM dyes may be toxic. While FM1-43 is not retained by paraformaldehyde fixation, FM1-43FX is a fixable version that displays identical excitation and emission spectra.

FM dyes are lipophilic labels generally absorbed by endocytosis with prolonged exposure. The internalized label then becomes generalized throughout the cell by membrane trafficking. The short incubation time used here insures that label is restricted to the neuromast sensory cells through a rapid uptake via the mechano-transduction channels. Membrane redistribution then transfers the dye throughout the cell over several minutes. The emission spectra for all FM dyes are extremely broad, a predominately red emission with a strong green signal, as well. It should also be noted that FM1-43 labeling seems to be inhibited by prior exposure to Syto dyes. This is being investigated further.

Dye	Paraformaldehyde Fixable	Specificity
Syto 24	Yes	Support and Sensory
Yo-Pro-1	Yes	Sensory
Lo-Pro-1	Yes	Sensory
To-Pro-3	Yes	Sensory
DASPEI	No	Mostly Sensory
Mitofluor Green	No	Sensory
Mitotracker Green	Yes	Sensory
Mitotracker Orange	Yes	Sensory
Mitotracker Red	Yes	Sensory
FM1-43	No	Sensory
FM1-43FX	Yes	Sensory

Working with fixed zebrafish.
Paraformaldehyde fixation of zebrafish induces strong autofluorescence in the green, red and far red channels. This autofluorescence may obscure all but exceptionally bright specific labels when viewed by widefield epifluorescence. Reducing the excitation intensity may assist in discernment of labels against background in fixed zebrafish when viewed with widefield epifluorescence. This autofluorescence can be reduced by using 2% paraformaldehyde or shorter fixation times.

High contrast, high resolution images from fixed fish require either confocal microscopy or deconvolution of image stacks collected from widefield epifluorescence.

The red Mitotrackers are based upon rhodamine derivatives. Anti-rhodamine antibodies from Molecular Probes are capable of labeling Mitotracker Red in zebrafish fixed with paraformaldehyde.