eFM488 Enhanced Fluorogenic Membrane Probe

V. 2.0

Cat. # MG15-50

Lot # **Amount: 50 nmoles**

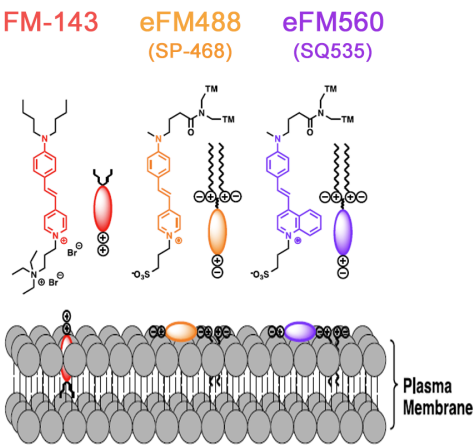
Upon arrival store at 4°C (desiccated)

See below for storage after reconstitution

Background

The eFM™ membrane probes are advanced versions of the widely used FM probes[1]. They contain modifications to the hydrophobic and ionic regions of the molecules to enhance their fluorogenic nature and retention within the plasma membrane (PM). In addition, the modifications allow 20 fold more photooxidation resistance[2]. Fluorogenic probes exhibit very low background because they only fluoresce when bound to their target location. The large Stokes shift makes eFM probes particularly superior for STED applications. eFM488 has been validated with multiple microscopy techniques including epifluorescence (widefield), confocal, and STED high resolution microscopy[2]. eFM488 is also known as SP-468[2].

Fig 1: Structure and mechanism of eFM™ probes



Legend: FM1-43 = original membrane dye. eFM488 = enhanced FM probe (Cat. # MG15). eFM555 = enhanced FM probe (Cat. # MG16). Integration with the PM is tighter for the two eFM probes compared to FM1-43 which enables enhancedstability in the PM.

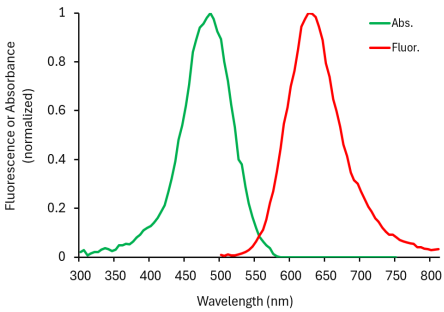
Material

The absorption wavelength maximum of eFM488 in DMSO is 483 nm, with an emission spectra maximum of 625 nm, an extinction coefficient of $50x10^3$ M^{-1} cm^{-1} and can be visualized using Ex: 488 nm, Em: 515-600 nm (or an FITC filter set Ex: 467-498, Em: 513-556 nm). eFM488™ is supplied as a lyophilized film. Avoid contact with the reagent by wearing appropriate personal protective equipment (PPE) and dispose according to local regulations.

Storage and Reconstitution

The lyophilized product is stable at 4°C (<10% humidity) for 6 months and should be protected from light. To reconstitute, briefly centrifuge to collect the product at the bottom of the tube. The 10 nmole size should be reconstituted with 100 µl of anhydrous DMSO, and the 50 nmole size should be reconstituted with 500 µl anhydrous DMSO to create a 100 µM stock solution for cell imaging. The solution should be stored at -20°C where it is stable for 12 months.

Fig 2: Absorbance and fluorescence spectra of eFM488



Legend: A 5 µM stock of eFM488 was prepared in DMSO and used to measure its absorbance and emission spectra.

Important technical notes

A. Diluted solutions of eFM™ in aqueous media will not precipitate, hence do not suffer from precipitation artifacts.

B. Extremely stable fluorescence, usually maintaining 90% fluorescence after 1h of exposure.

C. Efficiently stain membranes in cell-to-cell contacts.

D. eFM's large Stokes shifts are useful for stimulated emission depletion (STED) super resolution imaging.

E. eFM is non-toxic, and live cells can be returned to normal cell media following labeling, and relabeled after 3-4 days.

F. When co-labeling with antibodies that require permeabilization, limit the concentration of Triton-X to 0.05%.

G. eFM is fully compatible with 4% paraformaldehyde (PFA); however, 4% PFA partially permeabilizes the cell membrane so internalization of probes should be expected.

H. For tissues and small organisms, an initial labeling concentration of 8 µM (1:12.5 dilution) is recommended. For cell culture, an initial labeling concentration of 1 µM (1:100 dilution) is recommended depending on the application (Table 1).

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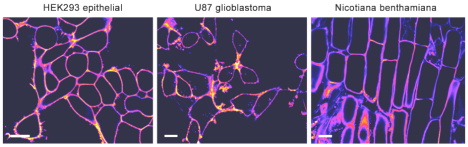
I. Homogeneity of tissue labeling can be optimized with a longer incubation at 4°C rather than relatively brief incubations at room temperature; however, both approaches can label plasma membranes.
J. Large Stokes shift fluorophores can enable multicolor imaging using a single excitation laser and separate emission filters, which simplifies optical setup and reduces spectral cross-talk in fluorescence microscopy.

Figure 3 - Fluorescence images of stained cells



Legend: Cells were stained with either 1 µM (HEK, U87) or 8 µM (N.b. tissue) for 10 min or 1 h respectively, and visualized by confocal microscopy. Scale bar = 15 µm.

Table 1 - Recommended initial concentrations of eFM488

	Live cells	Fixed cells	Tissue or small organisms
STED microscopy (µM)	0.25-1.0	1.0	4.0-8.0
Confocal microscopy (µM)	1.0	1.0	8.0

Note: Optimal conditions for efficient labeling should be determined for each cell line and application.

Application 1: Labeling the plasma membrane of live cells in culture and tissue sections.

Reagents
1. eFM488 (Cat. # MG15) 100 µM stock solution.
2. Semi-confluent cell culture in appropriate growth chamber.
3. Imaging medias: PBS, serum-free media or reduced serum media.

Equipment
1. Confocal microscope with Ex: 488 nm, Em: 515-600 nm (or an FITC filter set Ex: 467-498, Em: 513-556 nm).
2. Perform STED microscopy using Ex: 488 nm, 592 nm depletion. Collect emission in the 502 and 585 nm channels.
3. CCD Digital camera.

Method
1. Cells should be seeded onto imaging-appropriate glass or plastics and grown according to cell line requirements to semi-confluency.
2. Remove any cell culture media from your cells and replace with serum-free media or low serum media. Do not allow the cells to dry.
3. Prepare the probe solution by diluting 2 µl of eFM™ stock in 198 µl of media to create a 1.0 µM working solution.
4. Remove serum free media and add diluted probe solution to cells until they are completely covered.
5. Incubate cells for 10 minutes at room temperature. Incubation at 37°C can also be used, but it will accelerate potential endocytosis of probes.
6. No washing step is required prior to imaging, but media can be replaced with serum containing media at this point.
7. Proceed with imaging.

Notes:
1. Serum can reduce eFM™ staining efficiency. In lieu of serum removal, the concentration of eFM™ can be increased.
2. For fixed tissue sections up to 50 µm thick, use eFM at 8 µM and incubate for 1-4 h, observe during staining to identify optimal time.

Application 2: Labeling the plasma membrane of fixed cells in culture and tissue sections.

Reagents
1. eFM488 (Cat. # MG15).
2. Semi-confluent cells grown on acid-washed coverslips or similar.
3. Phosphate-buffered saline (PBS, 20 mM potassium phosphate pH 7.4, 150 mM NaCl).
4. Fixative solution (4.0 % paraformaldehyde in PBS).
5. Glass microscope slide.
6. Coverslip sealing solution.
7. EMS Fluoro-Gel mounting media (Cat. # 17985-10) or similar.

Equipment
1. Same as for live cell technique.

Method
1. Cells should be seeded onto imaging-appropriate glass or plastics and grown according to cell line requirements to semi-confluency.
2. Remove cell media and wash cells 1X-2X with PBS.
3. Fix cells for 10-15 minutes at room temperature with 4% paraformaldehyde (PFA) and 0.1% glutaraldehyde (GLT).
4. Remove excess PFA/GLT by washing cells with PBS 3X.
5. (Optional) If co-labeling, permeabilization can be performed at this point. Add 0.05% Triton-X 100 in PBS, followed by the primary and secondary antibody protocol according to the supplier.
6. Prepare the probe solution by diluting 100 µM eFM stock in PBS to create a 1.0 µM working solution (1 µl stock per 99 µl PBS), and mix thoroughly.
7. Exchange PBS wash for the eFM™ solution and incubate for 10 minutes at room temperature.
8. Remove eFM™ solution and wash cells twice with PBS.
9. If desired, place mounting media onto the microscope slide.
10. Apply cover slip cell-side down onto microscope slide.
11. If desired, apply coverslip sealing solution according to manufacturer's directions.
12. Proceed with imaging.

Note: For fixed tissue sections up to 50 µm thick, use eFM at 8 µM and incubate for 1 h.

Product Citations/Related Products
1. Betz, W. J. et al. 1992. Activity-dependent fluorescent staining and destaining of living vertebrate motor nerve terminals. J. Neurosci. 12 (2), 363−375.
2. Collot, M. et al. 2020. Molecular tuning of styryl dyes leads to… Bioconjugate Chem. 2020, 31, 875−883.

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