

FM® Lipophilic Styryl Dyes

Quick Facts

Storage upon receipt:

- Desiccate
- Protect from light

Introduction

FM dyes are lipophilic styryl compounds used in a wide variety of studies involving the plasma membrane and vesiculation. Molecular Probes offers the popular green-fluorescent FM® 1-43 and red-fluorescent FM® 4-64 dyes. Recently, we have added aldehyde-fixable (FX) analogs of these two dyes to the product line—FM® 1-43FX and FM® 4-64FX dyes, which utilize aliphatic amines for crosslinking by formaldehyde or glutaraldehyde. Additional analogs include FM® 2-10 dye, a more hydrophilic version of FM® 1-43 dye that allows a faster destaining rate for some quantitative applications, and FM® 5-95 dye, a slightly less lipophilic analog of FM® 4-64 dye.

The water-soluble FM® dyes, which are nontoxic to cells and virtually nonfluorescent in aqueous media, are believed to insert into the outer leaflet of the surface membrane where they become intensely fluorescent. This method of membrane labeling has been used to selectively visualize plasma membrane in cultured eukaryotic cells¹ and sea urchin eggs,² and to study endocytosis and exocytosis in eukaryotic cells^{3,4} and bacteria,^{5,6} vesicle trafficking in plant cells,⁷ vacuolarization in *Dictyostelium* amoebae,⁸ and sporulation in *Bacillus subtilis* bacteria.⁹ FM® dyes have shown utility in synaptosomal studies as well. In neurons that are actively releasing neurotransmitters, these dyes become internalized within the recycled synaptic vesicles, and the nerve terminals become brightly stained.¹⁰⁻¹³

Materials, Storage, and Handling

Though the amounts provided differ depending on the product (see *Compound Properties* below), each vial of FM® dye contains lyophilized material that can be dissolved in either water or DMSO to make a stock solution. The lyophilized dyes are stable at room temperature for 6 months if stored desiccated and protected from light. Stock solutions of the FX analogs are relatively unstable; they should be stored at $\leq -20^{\circ}\text{C}$ and discarded after two weeks.

Note: We are not aware of toxicity data for or health hazards of the FM® dyes. Treat the dyes with the same safety precautions as other chemicals with unknown toxicity, and dispose of them in accordance with local regulations.

Compound Properties

Cat #	Dye	Unit Size	MW	Ex	Em
T3163	FM® 1-43†	1 mg	611.55	510	626
T35356	FM® 1-43†	10 x 100 µg	611.55	510	626
F35355	FM® 1-43FX	10 x 100 µg	560.09	510	626
T7508	FM® 2-10	5 mg	555.44	506	620
T3166	FM® 4-64	1 mg	607.51	558	734
T13320	FM® 4-64	10 x 100 µg	607.51	558	734
F34653	FM® 4-64FX	10 x 100 µg	788.75	565	744
T23360	FM® 5-95	1 mg	565.43	560	734

† FM® 1-43 dye has excitation maximum (Ex) = 479 nm, emission maximum (Em) = 598 nm when bound to phospholipid bilayer membranes. Em = 565 nm when bound to synaptosomal membranes.

Fluorescence spectra are generated in solution in MeOH or CHCl₃; styryl dyes are generally nonfluorescent in water. Fluorescence emission maxima are pH dependent. Bound dyes are typically imaged with either a standard FITC filter set or TRITC filter set. Excitation and emission of styryl dyes are at shorter wavelengths in membrane environments than in reference solvents such as methanol (above). The difference is typically 20 nm for excitation and 80 nm for emission but varies considerably from one dye to another.

Plasma Membrane Labeling Protocol

The protocol below is intended to serve as a guideline for plasma membrane labeling of live, adherent cultured cells on coverslips, utilizing FM® 1-43, FM® 1-43FX, FM® 4-64, or FM® 4-64FX dye. Optimal conditions may vary depending upon the characteristics of the cells used. This protocol was optimized using highly confluent bovine pulmonary endothelial cells adherent to coverslips, and it has been confirmed to work for other common cell lines.

Because these dyes are quickly endocytosed, it is important to follow the temperature and time guidelines as closely as possible in order to slow down endocytosis and promote selective plasma membrane labeling and imaging. Endocytosis will likely occur within 10 minutes of staining.

Hanks' balanced salt solution (HBSS) without magnesium or calcium is suggested for this protocol (Invitrogen #14175079). The presence of magnesium or calcium apparently speeds up endocytosis of the dye, resulting in poor plasma membrane selectivity.

Staining

1.1 Prepare a working staining solution of 5 µg/mL dye in ice-cold HBSS; keep this staining solution on ice.

1.2 Remove the coverslip from the culture medium and quickly immerse it in the staining solution, *on ice*, for 1 minute. The plasma membranes will stain quickly. If fixation is desired for the FX dyes, proceed to the fixation protocol below.

1.3 Remove the coverslip from the staining solution. Mount on a microscope slide, seal with paraffin, keep on ice, and image

immediately. For best results, image the sample in staining solution without washing.

Fixation (for FX dyes only)

2.1 Fix the stain with ice-cold 4% formaldehyde in HBSS; place on ice for 10 minutes.

2.2 Rinse the coverslip three times with HBSS.

2.3 Mount the coverslip in HBSS, seal, and image.

References

1. Cell Physiol Biochem 15, 159 (2005); 2. J Cell Biol 131, 1183 (1995); 3. J Cell Biol 149, 397 (2000); 4. Am J Physiol 276, 376 (1999); 5. Am J Physiol Cell Physiol 282, 1042 (2002); 6. J Cell Biol 128, 779 (1995); 7. J Microsc 214, 159 (2004); 8. J Cell Biol 121, 1311 (1993); 9. Proc Natl Acad Sci USA 96, 14553 (1999); 10. Nature 392, 497 (1998); 11. Science 255, 200 (1992); 12. Annu Rev Physiol 60, 347 (1998); 13. Neuron 41, 755 (2004).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
F35355	FM® 1-43FX *fixable analog of FM® 1-43 membrane stain*	10 x 100 µg
F34653	FM® 4-64 FX *fixable analog of FM® 4-64 membrane stain*	10 x 100 µg
T3163	N-(3-triethylammoniumpropyl)-4-(4-(dibutylamino)styryl) pyridinium dibromide (FM® 1-43)	1 mg
T35356	N-(3-triethylammoniumpropyl)-4-(4-(dibutylamino)styryl) pyridinium dibromide (FM® 1-43) *special packaging*	10 x 100 µg
T7508	N-(3-triethylammoniumpropyl)-4-(4-(diethylamino)styryl) pyridinium dibromide (FM® 2-10)	5 mg
T3166	N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl)hexatrienyl)pyridinium dibromide (FM® 4-64)	1 mg
T13320	N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl)hexatrienyl)pyridinium dibromide (FM® 4-64) *special packaging*	10 x 100 µg
T23360	N-(3-trimethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl)hexatrienyl)pyridinium dibromide (FM® 5-95)	1 mg

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

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