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- Mindermengenzuschlag
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Revised: May 11, 2018

Product Information

Fixable Nerve Terminal Dyes

Catalog Number	Product	m[a]	n[a]	MW	Ex/Em (MeOH)	Ex/Em (membranes)
70024	AM1-43	3	1	559.5	510/625 nm	~480/600 nm see Fig. 3
70038	AM1-44	4	1	587.5		
70036	AM2-10	1	1	503.5		
70051	AM3-25	17	1	952.83		
70053	HM1-43	3	1	602.1		
70025	AM4-64	1	3	555.5	543/- nm[b]	~510/750 nm[c] see Fig. 4
70039	AM4-65	3	3	844.85		
70050	AM4-66	4	3	872.85		

a. See Figure 1.
b. Emission in MeOH is too weak to measure.
c. Excitation/emission settings of 515/640 nm have been used for detection of yeast vacuole staining with SynaptoRed C2 (FM4-64) (Ref. 1); these settings should be applicable to AM4-64 and AM4-65.

Unit Size: 1 mg

Storage and Handling

Store desiccated at 4°C or below. Protect from light, especially in solution. Product is stable for at least 12 months from date of receipt when stored as recommended. Dyes are soluble in water. Stock solutions can be prepared at 10 mM and stored at 4°C or -20°C for six months or longer.

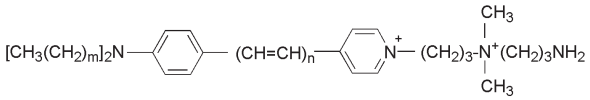

Figure 1. General structure of AM fixable nerve terminal dyes, where m = 0-17 and n = 1 or 3.

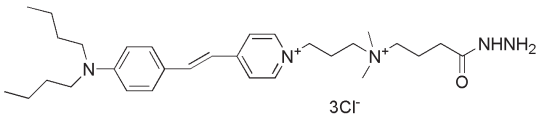

Figure 2. Structure of HM1-43 fixable nerve terminal dye.

Product Description

Nerve terminal probes are a series of fluorescent cationic styryl dyes developed to follow synaptic activities at neuromuscular junctions or synapses. These dyes typically have a lipophilic tail (two carbon chains) at one end and a highly hydrophilic, cationically charged head group at the other end. These nerve terminal probes were originally called FM® dyes, and are available from Biotium under the trademark names of SynaptoGreen™ and SynaptoRed™. SynaptoGreen™ probes are dyes with a single double bond (n = 1) while SynaptoRed probes are dyes with three double bonds (n = 3). A nerve terminal dye is named as either SynaptoGreen™ or SynaptoRed™ followed by a carbon number designating the length of the lipophilic tail. AM dyes have similar structures, except that they possess an aldehyde-fixable amine on the positively-charged head group (Fig. 1). HM1-43 has a hydrazide group instead of an amine group (Fig. 2), which is more reactive with formaldehyde. Because the hydrazide group is neutral, HM1-43 is more lipophilic than AM1-43.

Cationic styryl dyes are believed to function by staining synaptic vesicles in an activity-dependent fashion. In the presence of cells or tissue preparations, the dyes partition between the aqueous phase, where the dyes are virtually non-fluorescent, and the outer leaflet of the cell surface membranes, where the dyes insert the lipophilic end into the membranes and become intensely fluorescent. During endocytosis following nerve stimulation, the dyes become trapped inside the vesicles. Thus, after washing off the dyes on the cell surface, the fluorescent signal is proportional to the number of newly formed vesicles. On the other hand, during exocytosis, the dyes are released from the vesicles along with neurotransmitters, causing a decrease in fluorescent signal. As a result, the change in fluorescent intensity reflects the amount of endocytosis/exocytosis or synaptic activity. The rate of fluorescence increase during endocytosis, the “on-rate”, and the rate of fluorescence decrease during exocytosis, the “off-rate”, vary from dye to dye. In general, dyes with longer lipophilic tails and more double bonds have a higher affinity toward membrane and thus a higher on-rate and lower off-rate.

Some styryl dyes can enter cells through ion channels. AM3-25 is the fixable analog of SynaptoGreen™ C18, which has a long carbon chain and cannot pass through ion channels. This dye has been used as a control to distinguish mechanisms of dye uptake (2).

When using nerve terminal dyes, one frequent problem researchers encounter is background fluorescence due to nonspecific membrane staining. Although most of the background fluorescence can be removed by repeated washing, the problem is still significant with dyes that have a longer tail or more double bonds, particularly when the dyes are used in tissue preparations. Biotium offers various background reducing agents for use with nerve terminal dyes. ADVASEP-7 is a sulfonated beta-cyclodextrin that helps to remove dye during washing (3). SCAS quenches fluorescence of membrane-bound dye without the need for repeated wsh steps (4). Sulforhodamine 101 is a red fluorescent dye that quenches background fluorescence from green nerve terminal dyes by FRET (5). We also offer Nerve Terminal Staining Kits that include dyes and background reducing agents (see Related Products).

References

1. Vida, TA and Emr, SD. J. Cell Biol 128, 779(1995).
2. Meyers, JR, et al. J Neurosci., 23, 4054(2003).
3. Kay, AR, et al. Neuron 24, 809 (1999).
4. Höltje, M, et al. J. Biol. Chem. 283(14):9289(2008)
5. Pyle, JL, et al. Neuron 24, 803 (1999).

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Assay Protocol

The following is an example of a protocol for nerve terminal staining of cultured neurons on coverslips, followed by fixation and immunostaining. Nerve terminal dyes also can be used to label endocytic vesicles in non-neuronal cell types. Staining can be performed at 4°C for selective labeling of the plasma membrane; at room temperature or 37°C, endocytosis of the dye generally occurs within 10 minutes. Buffers other than Tyrode solution may be used. The addition of the sodium channel blocker tetrodotoxin (TTX) is optional, its purpose is to block action potentials and prevent synaptic vesicle release after staining. Optimal protocols for specific applications may need to be determined by the user; see reference 3 for examples of protocols for staining brain slices and other tissue samples.

1. Dilute the dye to a final concentration of 4 uM in 50 mM Tyrode solution. Place the coverslip with your cells in this solution for 1 minute at room temperature. Use enough solution to completely submerge the cells.
2. Transfer the coverslip to Tyrode + 0.5 uM tetrodotoxin (TTX, catalog no. 00061) solution for 1 minute at room temperature.
3. Wash the coverslip several times in Tyrode + 0.5 uM TTX at room temperature.

   Note: to reduce background, 1 mM ADVASEP-7 (catalog no. 70029) can be added to the wash solution. Alternatively, SCAS (catalog no. 70037) can be used to quench background without repeated washes. Incubate the coverslip for 4 minutes at room temperature in Tyrode + TTX + 0.5 mM SCAS.
4. Transfer the coverslip to fixation solution (4% formaldehyde, 4% sucrose, 1 uM TTX in PBS) for 20 minutes at room temperature.
5. Transfer the coverslip directly to pre-chilled 0.01% Triton X-100 in PBS for 12 minutes at 4°C.
6. Wash 3 x 1 minute with cold PBS.
7. Stain with primary antibody in 10% serum/PBS for 3 hours at 4°C. The concentration of the antibody should be double of what you would use for regular immunofluorescence staining.
8. Wash 3 x 1 minute with cold PBS.
9. Stain the preparation with secondary antibody at the concentration you would normally use for immunofluorescence staining in 2% serum/PBS for 40 minutes at 4°C.
10. Wash as in step 8.
11. Mount the coverslip in PBS and image.

    Note: for dyes with Ex/Em (in membranes) at 480/600 nm, 50 uM sulforhodamine 101 (catalog no. 80101) can be included in the mounting buffer to quench extracellular fluorescence.

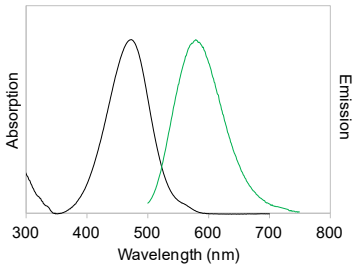

Figure 3. Absorption and emission spectra of SynaptoGreen™ C4 in liposomes. Spectra for AM1-43, AM1-44, AM2-10, AM3-25, and HM1-43 are similar.

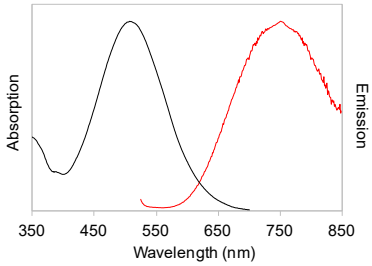

Figure 4. Absorption and emission spectra of SynaptoRed™ C2 in liposomes. Spectra for AM4-64 and AM4-65 are similar.

Related Products

Catalog number	Product
70042	SynaptoGreen™ C1
70044	SynaptoGreen™ C2 (equivalent to FM®2-10)
70023	SynaptoGreen™ C3
70020	SynaptoGreen™ C4 (equivalent to FM®1-43)
70046	SynaptoGreen™ C5 (equivalent to FM®4-84)
70048	SynaptoGreen™ C18 (equivalent to FM®1-84)
70040	SynaptoRed™ C1
70021	SynaptoRed™ C2 (equivalent to FM®4-64)
70028	SynaptoRed™ C2M (equivalent to FM®5-95)
70029	ADVASEP-7
70037	SCAS
80101	Sulforhodamine 101
70030	Nerve Terminal Staining Kit I 5 x 1 mg SynaptoGreen™ C4 and 250 mg ADVASEP-7
70031	Nerve Terminal Staining Kit II (A) 1 mg AM1-43 and 100 mg ADVASEP-7
70031-1	Nerve Terminal Staining Kit II (B) 1 mg AM1-43 and 100 mg SCAS
70032	Nerve Terminal Staining Kit III 5 x 1 mg SynaptoGreen™ C4 and 100 mg Sulforhodamine 101
70034	Nerve Terminal Staining Kit V 5 x 1 mg SynaptoRed™ C2 and 250 mg ADVASEP-7
00060	Tetrodotoxin, citrate-free
00061	Tetrodotoxin, with citrate
00010	α-Bungarotoxin
00019	β-Bungarotoxin

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® dye bungarotoxins, antibodies, and other conjugates, calcium and other ion indicator dyes, apoptosis detection reagents, and other fluorescent probes and kits for cell biology research.