Fluorescent nerve terminal dyes

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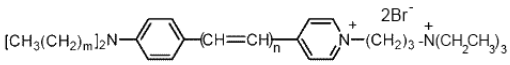
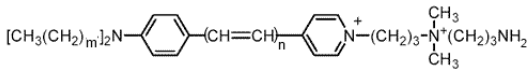
Nerve terminal probes are a series of fluorescent cationic styryl dyes developed to follow synaptic activity at neuromuscular junctions or synapses. These dyes typically have a lipophilic tail (two carbon chains) at one end and a highly hydrophilic, cationically charged head group at the other end as illustrated by the general structure below:



SynaptoGreen and SynaptoRed dyes
m = 0-17; n = 1-3

where m is the number of carbons in the lipophilic tail and n is the number of double bonds linking the two aromatic rings in the dye. These nerve terminal probes were originally called FM™ dyes, and are now they are available from Biotium under the trademark names SynaptoGreen™ and SynaptoRed™, depending on the wavelength of the dyes. 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. SynaptoGreen C4 is equivalent to FM1-43, while SynaptoRed C2 is the same as FM4-64. A comprehensive list of nerve terminal dyes is shown in Table 1.

Biotium has also developed a series of fixable nerve terminal dyes, known as "AM" dyes, which are closely related to the FM dyes. The primary difference between the fixable and non-fixable dyes is that the formers have an additional formaldehyde-fixable amino group attached to the positively-charged head group of the dyes:



AM™ dyes: m = 1-17; n = 1-3

AM2-10, AM1-43 and AM1-44 are derivatives of SynaptoGreen that have relatively shorter wavelengths (Fig. 1), while AM4-64 and AM4-65 are based on SynaptoRed, and have relatively longer wavelengths (Fig. 2; Renger et al. 2001).

Nerve terminal dyes stain synaptic vesicles in an activity-dependent fashion. When added to cells or tissue preparations, the dyes partition between the aqueous phase, where they are virtually non-fluorescent, and the outer leaflet of the plasma membrane, where the lipophilic tails of the dyes insert into the membrane, causing the dyes to become intensely fluorescent. Following nerve stimulation, the dye molecules become trapped inside endocytic vesicles. Thus, after washing the dyes from 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 fluorescence 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. AM dyes, which have an additional amine, tend to be even more water-soluble and thus higher off-rate and lower on-rate than the corresponding FM dye counterparts that have the same lipophilic tail length (Table 1).

A common problem encountered with nerve terminal dyes is background fluorescence due to residual membrane staining after washing. Although most of the surface fluorescence can be removed by repeated washes, the problem is still significant with dyes that have a longer tail or more double bonds, particularly in tissue preparations. To reduce the background fluorescence, we offer three quencher or dye-clearing agents. ADVASEP-7 (Kay et al. 1999), a sulfonated β-cyclodextrin, forms a water soluble inclusion complex with SynaptoGreen C4 that can be removed more effectively by washing. Biotium's unique quencher, SCAS, reduces background fluorescence as soon as it is added to the preparation without the need for washing. Sulforhodamine 101 has also been used to reduce SynaptoGreen C4 background staining via fluorescent resonance energy transfer (FRET) (Pyle et al. 1999). We offer these quencher/dye-clearing agents as individual products and as kits as well that contain both the dyes and the quencher/dye-clearing agents (Table 2).

References:
Kay, A.R., et al. Neuron 24, 809 (1999).
Pyle, J.L., et al. Neuron 24, 803 (1999).
Renger, J.J., et al., Neuron 29, 469 (2001).
Vida, T.A. and Emr, S.D. J Cell Biol 128, 779 (1995).

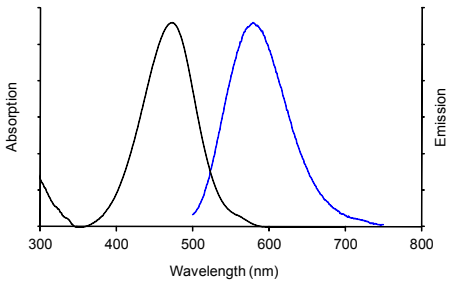


Figure 1. Absorption and emission of SynaptoGreen™ C4 (FM1-43) in liposomes. Spectra for AM1-43, AM1-44, AM2-10, and other SynaptoGreen dyes are similar.

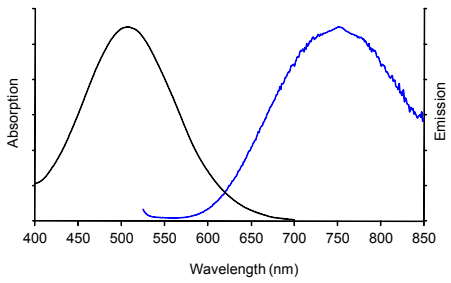


Figure 2. Absorption and emission of SynaptoRed™ C2 (FM4-64) in liposomes. Spectra for AM4-64, AM4-65, AM4-66, and other SynaptoRed dyes are similar.

Table 1. Properties of Fluorescent Nerve Terminal Probes

Cat. No.	Product Name	Abs/Em (nm)[a] (in MeOH)	Abs/Em (nm)[c] (in membranes)	m	n
70024 (1 mg)	AM1-43	510/625	~480/600	3	1
70038 (1 mg)	AM1-44	510/625	~480/600	4	1
70036 (1 mg)	AM2-10	510/625	~480/600	1	1
70051 (1 mg)	AM3-25	510/625	~480/600	17	1
70025 (1 mg)	AM4-64	543/b	~510/750	1	3
70039 (1 mg)	AM4-65	543/b	~510/750	3	3
70042 (5 mg), 70043 (5 x 1 mg)	SynaptoGreen C1	~500/615	~480/600	0	1
70044 (5 mg), 70045 (5 x 1 mg)	SynaptoGreen C2 (FM2-10)	505/620	~480/600	1	1
70023 (5 mg), 70026 (5 x 1 mg)	SynaptoGreen C3	~510/625	~480/600	2	1
70020 (5 mg), 70022 (5 x 1 mg)	SynaptoGreen C4 (FM1-43)	510/625	~480/600	3	1
70046 (5 mg), 70047 (5 x 1 mg)	SynaptoGreen C5 (FM1-84)	510/625	~480/600	4	1
70048 (5 mg), 70049 (5 x 1 mg)	SynaptoGreen C18 (FM3-25)	510/625	~480/600	17	1
70040 (5 mg), 70041 (5 x 1 mg)	SynaptoRed C1	~540/b	~510/750	0	3
70021 (5 mg), 70027 (5 x 1 mg)	SynaptoRed C2 (FM4-64)	543/b	~510/750	1	3
70019 (5 mg), 70028 (5 x 1 mg)	SynaptoRed C2M[d] (FM5-95)	543/b	~510/750	1	3

[a] The spectra of styryl dyes are known to undergo a large blue shift (30 to 40 nm)when going from polar media (MeOH) to nonpolar media (membranes). [b] emission spectra are too weak to measure in MeOH. [c] Data were obtained with liposome suspension to mimic cell membrane environment. Excitation/emission wavelength setting at 515/640 nm has been used for detection of yeast vacuole membrane staining with SynaptoRed C2 (FM4-64) (Vida and Emr 1995), and this setting should also be applicable to SynaptoRed C1, AM4-64, AM4-65 and AM4-66. [d] The positively-charged end of SynaptoRed C2M is a trimethylammonium group instead of a triethylammonium group.

Table 2. Nerve Terminal Staining Kits

Cat. No.	Product Name	Kit Contents
70030	Nerve Terminal Staining Kit I	5 x 1 mg SynaptoGreen C4 (70022) 250 mg ADVASEP-7 (70029)
70031	Nerve Terminal Staining Kit II (A)	1 mg AM1-43 (70024) 100 mg ADVASEP-7 (70029)
70031-1	Nerve Terminal Staining Kit II (B)	1 mg of AM1-43 (70024) 100 mg SCAS (70037)
70032	Nerve Terminal Staining Kit III	5 x 1 mg SynaptoGreen C4 (70022) 100 mg Sulforhodamine 101(80101)
70034	Nerve Terminal Staining Kit V	5 x 1 mg SynaptoRed C2 (70027) 250 mg ADVASEP-7 (70029)
70035	Nerve Terminal Staining Kit VI	5 x 1 mg SynaptoRed C2 (70027) 100 mg sulforhodamine 101 (80101)