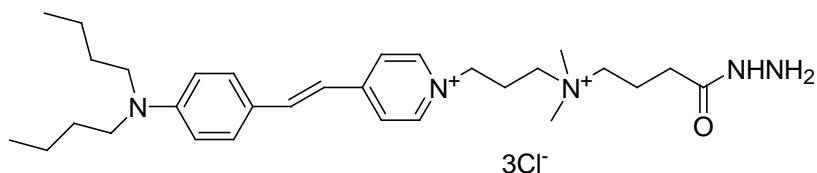


PRODUCT AND SAFETY DATA SHEET

PRODUCT NAME: HM1-43

CATALOG #: 70053

MOLECULAR INFORMATION: C₃₀H₄₉Cl₃N₅O
MW: 602.1

**PROPERTIES:**

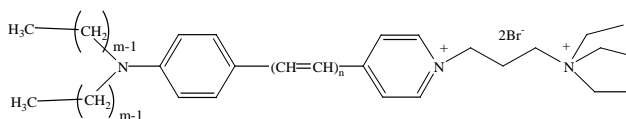
Color & Form	Dark red solid
Purity	98% by TLC
Solubility	Soluble in water
Absorption/Emission	510/625 nm(in MeOH); 480/600 nm(in membranes)
Extinction Coefficient	~50,000

STORAGE AND HANDLING:

Store desiccated at ≤ 4 °C. Protect from light, especially in solution. Stock solution may be prepared in water and stored at 4 °C or -20 °C for at least one year.

APPLICATION:

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 as illustrated by the general structure below:



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 now they 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

**APPLICATION:
(CONTINUED)**

SynaptoRed followed by a carbon number designating the length of the lipophilic tail. As a result, our SynaptoGreen C4 is equivalent to FM1-43, while our SynaptoRed C2 is the same as FM4-64.

Biotium has the largest collection of high quality nerve terminal dyes available to you at a fraction of the prices of our competitor's products. A comprehensive list of nerve terminal dyes are shown in Table 1.

Biotium has developed **AM1-43** (*Neuron*, **29**, 469(2001)) as a fixable version of SynaptoGreen C4 (FM1-43). AM1-43 has the same absorption/emission spectra and the same lipophilic tail as SynaptoGreen C4 does except that the former has an additional amine group that makes the dye fixable with a standard aldehyde fixative agent. In conjunction with the use of AM1-43, Biotium offers a quencher agent called SCAS that efficiently removes background fluorescence when added to the preparation without the need for repeated washing (See below). In addition, we have also developed other fixable dyes based other SynaptoGreen and SynaptoRed dyes. A complete list of fixable AM dyes is presented in the Table below. More recently, we developed a new fixable dye called HM1-43 (cat# 70053). This new dye is very similar to AM1-43 except that the amino group of AM1-43 is replaced by a hydrazide group, which is expected to be more fixable than the amino group.

The highly water soluble 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 nonfluorescent, and the outer leaflet of the cell surface membranes, where the dyes insert the lipophilic end into the membranes and are 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 signals. 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.

When using these nerve terminal dyes, one frequent problem researchers encounter is the background fluorescence due to nonspecific membrane staining. Although most of the background fluorescence can be removed by repeated washings with a buffer, 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. To reduce the background fluorescence, we offer three quencher/dye-clearing agents. **ADVASEP-7** (Kay, A.R., et al. *Neuron* **24**, 809(1999)), a sulfonated β -cyclodextrin, forms a water soluble inclusion complex with SynaptoGreen C4, thus leaving the dye in the aqueous phase. The dye-dextrin inclusion complex is actually much more fluorescent than the free dye in solution. However, repeated washings remove the complex and thus lower the background fluorescence. Biotium's unique quencher, SCAS, reduces background fluorescence as soon as it is added to the preparation without the need for repeated washings. Sulforhodamine 101 has also been used to reduce SynaptoGreen C4 background staining via fluorescent resonance energy transfer(FRET) (Pyle, J.L., et al. *Neuron* **24**, 803(1999)). We offer these quencher/dye-clearing agents as individual products and also kits that contain both the dyes and the quencher/dye-clearing agents.

*FM is a trademark of Molecular Probes, Inc.

**APPLICATION:
(CONTINUED)**Ref.: 1) *Neuron*, **29**, 469 (2001); 2) *The Journal of Neuroscience*, **23**(10), 4054(2003); 3) *Cell Calcium*, **39**, 57 (2006)**Table 1. Properties of Fluorescent Nerve Terminal Probes**

Cat. #	Product Name	Equivalent FM™ dye	$\lambda_{abs}/\lambda_{em}$ (nm, in MeOH) ^a	$\lambda_{abs}/\lambda_{em}$ (nm, in membranes)	Fixability
70024	AM1-43	none	510/625	479/598	Yes
70038	AM1-44	none	510/625	479/598	yes
70036	AM2-10	none	502/625	479/598	Yes
70051	AM3-25	None	502/625	480/600	yes
70025	AM4-64	None	543/b	c	yes
70039	AM4-65	None	543/b	c	yes
70050	AM4-66	None	543/b	c	yes
70053	HM1-43	none	510/625	480/600	yes
70042, 70043	SynaptoGreen C1	none	510/625	479/598	No
70044, 70045	SynaptoGreen C2	FM2-10	505/620	d	No
70023, 70026	SynaptoGreen C3	none	510/625	d	No
70020, 70022	SynaptoGreen C4	FM1-43	510/625	479/598	No
70046, 70047	SynaptoGreen C5	FM1-84	510/625	d	No
70048, 70049	SynaptoGreen C18	FM3-25	510/625	479/598	possible
70040, 70041	SynaptoRed C1	none	543/b	c	No
70021, 70027	SynaptoRed C2	FM4-64	543/b	c	No
70019, 70028	SynaptoRed C2M	FM5-95	543/b	510-750	No
<p>^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 AM4-64, SynaptoRed C1 and SynaptoRed C2 have similar absorption spectra and their emission spectra are too weak to measure in MeOH. ^c Data have not been determined yet. However, excitation/emission wavelength setting at 515/640 nm has been used for detection of yeast vacuole membrane staining with SynaptoRed C2 (FM4-64) [J. Cell. Biol. 128, 779(1995)], and this setting should also be applicable to SynaptoRed C1. ^d Data are not available, but expected to be similar to that for AM1-43 or SynaptoGreen C4.</p>					

TOXICITY: Unknown.

FIRST AID:	Potentially harmful. Avoid prolonged or repeated exposure. Avoid getting in eyes, on skin, or on clothing. Wash thoroughly after handling. If eye or skin contact occurs, wash affected areas with plenty of water for 15 minutes and seek medical advice. In case of inhaling or swallowing, move individual to fresh air and seek medical advice immediately.
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Disclaimer: <i>Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use. Biotium is not liable for any damage resulting from handling or contact with this product.</i>
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