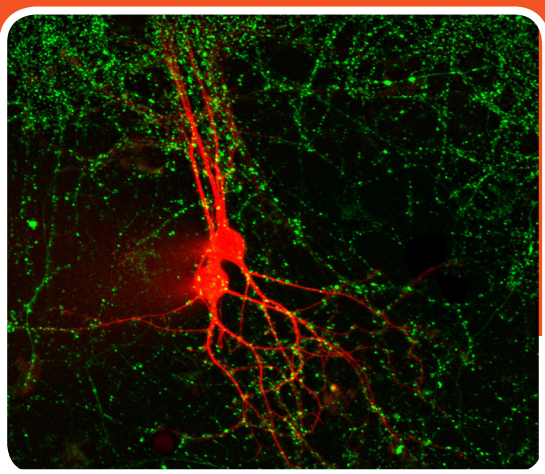

Nerve Terminal Probes

Fluorescent dyes for labeling and tracking synaptic vesicles

SynaptoGreen™ & SynaptoRed™

- Activity-dependent fluorescent synaptic vesicle dyes
- Aldehyde-fixable AM and HM versions
- Nerve terminal staining kits with background reducing reagents

SynaptoGreen™ and SynaptoRed™

Nerve terminal probes (originally called FM® dyes) are sold by Biotium under the trademark names SynaptoGreen™ and SynaptoRed™, depending on their fluorescence emission. They have a lipophilic tail at one end and a highly hydrophilic, cationically charged head group at the other end (Figure 1), 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.

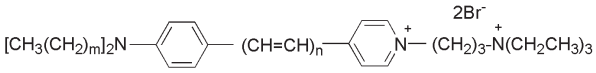

Figure 1. General structure of SynaptoGreen and SynaptoRed dyes. m = 0-17; n = 1-3.

Nerve terminal dyes stain synaptic vesicles in an activity-dependent fashion. They are virtually non-fluorescent in solution, but when added to cells, the lipophilic tails of the dyes insert into the plasma membrane, causing the dyes to become intensely fluorescent. Following nerve stimulation, the dye molecules are internalized in endocytic vesicles ("on-rate"). During exocytosis, the dyes are released from the vesicles along with neurotransmitters, causing a decrease in fluorescence signal ("off-rate"). In general, dyes with longer lipophilic tails and more double bonds have a higher affinity for membrane and thus a higher on-rate and lower off-rate. AM dyes, which have an additional amine (Figure 2), tend to be even more water-soluble and thus have a higher off-rate and lower on-rate than the corresponding FM dye. Some dyes can enter cells through ion channels; SynaptoGreen C18 and AM3-25 are high molecular weight dyes that cannot pass through ion channels that have been used as controls to distinguish mechanisms of dye uptake.

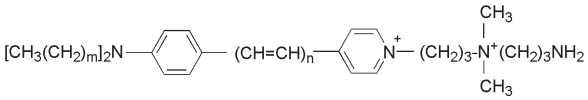

Figure 2. General structure of AM fixable nerve terminal dyes. m = 0-17; n = 1-3.

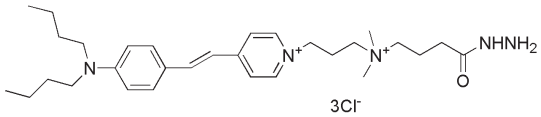

Figure 3. HM1-43 fixable nerve terminal dye.

Fixable synaptic vesicle dyes

AM dyes and HM dyes are fixable nerve terminal dyes. After staining with these dyes, cells can be fixed and permeabilized for subsequent immunostaining. The AM dyes have an aldehyde-fixable amino group attached to the positively-charged head group of the dyes. HM1-43 is similar except that the amino group is replaced by a hydrazide, which is more reactive with aldehyde fixatives. Because the hydrazide group is neutral, HM1-43 (Figure 3) is more lipophilic than AM1-43.

Background quenchers and Nerve Terminal Staining Kits

A common problem encountered with nerve terminal dyes is background fluorescence due to residual membrane staining, even after extensive washing. To reduce this background fluorescence, we offer three quencher or dye-clearing agents. ADVASEP-7, 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 quenches SynaptoGreen C4 background staining via fluorescent resonance energy transfer (FRET). We offer these reagents as individual products and in kits with dyes and the quencher/dye-clearing agents.

Biotium

SynaptoGreen™, SynaptoRed™, and Fixable Nerve Terminal Dyes

Cat. #	Size	Product name	m*	n*	Ex/Em in membranes	Fixable?
70042, 70043	5 mg, 5 x 1 mg	SynaptoGreen™ C1	0	1	~480/600 nm	No
70044, 70045	5 mg, 5 x 1 mg	SynaptoGreen™ C2 (equivalent to FM®2-10)	1	1	~480/600 nm	No
70023, 70026	5 mg, 5 x 1 mg	SynaptoGreen™ C3	2	1	~480/600 nm	No
70020, 70022	5 mg, 5 x 1 mg	SynaptoGreen™ C4 (equivalent to FM®1-43)	3	1	~480/600 nm	No
70046, 70047	5 mg, 5 x 1 mg	SynaptoGreen™ C5 (equivalent to FM®1-84)	4	1	~480/600 nm	No
70048, 70049	5 mg, 5 x 1 mg	SynaptoGreen™ C18 (equivalent to FM®3-25)	17	1	~480/600 nm	No
70024	1 mg	AM1-43	3	1	~480/600 nm	Yes
70038	1 mg	AM1-44	4	1	~480/600 nm	Yes
70036	1 mg	AM2-10	1	1	~480/600 nm	Yes
70051	1 mg	AM3-25	17	1	~480/600 nm	Yes
70053	1 mg	HM1-43	3	1	~480/600 nm	Yes
70040, 70041	5 mg, 5 x 1 mg	SynaptoRed™ C1	0	3	~510/750 nm	No
70021, 70027	5 mg, 5 x 1 mg	SynaptoRed™ C2 (equivalent to FM®4-64)	1	3	~510/750 nm	No
70019, 70028	5 mg, 5 x 1 mg	SynaptoRed™C2M** (equivalent to FM®5-95)	1	3	~510/750 nm	No
70025	1 mg	AM4-64	1	3	~510/750 nm	Yes
70039	1 mg	AM4-65	3	3	~510/750 nm	Yes
70050	1 mg	AM4-66	4	3	~510/750 nm	Yes

*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; see other side for general dye structures.
**The positively-charged end of SynaptoRed C2M is a trimethylammonium group.

Nerve Terminal Staining Kits

Cat. #	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)

Other neuronal stains (visit www.biotium.com)

- PathoGreen™ Histofluorescent Stain for neurodegeneration
- Congo Red for colorimetric or fluorescent staining of amyloid plaques
- DCDAPH for near-IR detection of Aβ1-42 aggregates and β amyloid
- Thioflavin T, cell permeable fluorescent amyloid probe

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