Revised: November 1, 2016

Product Information

Nerve Terminal Staining Kit IV

Catalog Number: 70030

Kit Contents

Component	Size
70027 SynaptoRed™ C2	5 x 1 mg
70029 ADVASEP-7	250 mg

Storage and Handling

Store desiccated at 4°C or below. Protect SynaptoRed C2 from light, especially in solution. Components are stable for at least 12 months from date of receipt when stored as recommended. SynaptoRed C2 and ADVASEP-7 are soluble in water. To prepare 10 mM SynaptoRed C2, dissolve 1 mg in 164 uL dH_2O. To prepare 100 mM ADVASEP-7, dissolve 250 mg in 1.16 mL dH_2O. Stock solutions can be stored at 4°C or -20°C for six months or longer.

Molecular information

SynaptoRed C2

MW: 608

Formula: $C_{30}H_{45}Br_2N_3$

See Fig. 1

ADVASEP-7

Average MW: 2163

Formula: $C_{42}H_{70-n}O_{35}(C_4H_8SO_3Na)_n$•hydrate where n = Average Degree of Substitution 6.5

Spectral Properties

SynaptoRed C2

Abs/Em 543/- nm (in MeOH); 510/750 nm (in membranes) (Fig. 2)

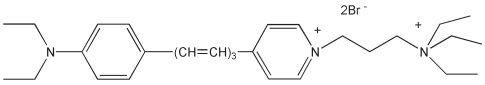

Figure 1. Structure of SynaptoRed C2

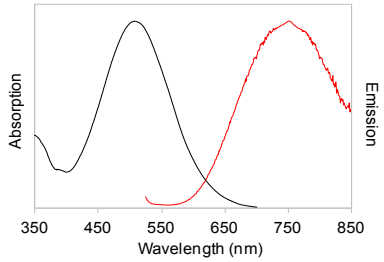

Figure 2. Absorption and emission spectra of SynaptoRed C2 (also known as FM4-64) in liposomes.

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. SynaptoRed C2 is equivalent to FM®4-64. Biotium also offers AM dyes, which are aldehyde-fixable analogs of SynaptoGreen and SynaptoRed (see related products).

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.

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. Washing with sulfonated beta-cyclodextrin ADVASEP-7 helps to remove dye from the cell surface and reduces background (1).

Biotium offers additional nerve terminal staining kits with other pairings of nerve terminal dyes and background reducing agents (see Related Products).

References

1. Kay, AR, et al. Neuron 24, 809 (1999).

Assay Protocol

The following is an example of a protocol for nerve terminal staining of cultured neurons on coverslips. 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 1 for examples of protocols for staining brain slices and other tissue samples.

1. Dilute SynaptoRed C2 stock solution to a final concentration of 4 uM in 50 mM Tyrode solution (for example, 1 uL 10 mM dye per 2.5 mL 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 for 5 minutes at room temperature with Tyrode + 0.5 uM tetrodotoxin + ADVASEP-7. The typical concentration of ADVASEP-7 working solution is 1 mM (for example, 10 uL of 100 mM ADVASEP-7 per mL solution).
4. Rinse the coverslip twice with Tyrode + 0.5 uM tetrodotoxin.
5. Mount the coverslip in Tyrode + 0.5 uM tetrodotoxin and image.

Note: SynaptoRed C2 is not fixable. For fixable staining, we offer AM4-64, which is an aldheyde-fixable analog of SynaptoRed C2 (see related products).

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)
70024	AM1-43
70038	AM1-44
70036	AM2-10
70051	AM3-25
70025	AM4-64
70039	AM4-65
70050	AM4-66
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
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.