

# Product Information

## Nerve Terminal Staining Kit V

**Catalog Number:** 70034

### Kit Contents

Catalog no.	Product	Unit 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 µL dH<sub>2</sub>O. To prepare 100 mM ADVASEP-7, dissolve 250 mg in 1.16 mL dH<sub>2</sub>O. Stock solutions can be stored at 4°C or -20°C for six months or longer.

### SynaptoRed™ C2

**Molecular Information:** C<sub>30</sub>H<sub>45</sub>Br<sub>2</sub>N<sub>3</sub>

**CAS number:** 162112-35-8

**Molecular Weight:** 608

**Color and Form:** Dark purple solid

**Solubility:** Soluble in water

**Absorption/Emission:** 543/- nm (in MeOH); 510/750 nm (in membranes)

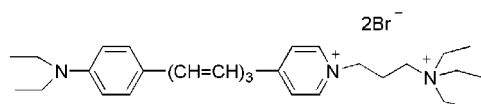


Figure 1. Structure of SynaptoRed™ C2

### ADVASEP-7

**Molecular Information:** C<sub>42</sub>H<sub>70-n</sub>O<sub>35</sub>(C<sub>4</sub>H<sub>8</sub>SO<sub>3</sub>Na)<sub>n</sub>•hydrate

where n = Average Degree of Substitution 6.5

**Molecular Weight:** ~2163 (average)

**Color and Form:** White solid

**Solubility:** Soluble in water

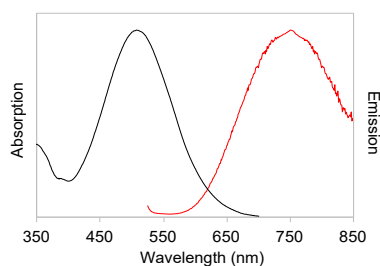


Figure 2. Absorption and emission spectra of SynaptoRed™ C2 (also known as FM@4-64) in liposomes. SynaptoRed™ C2 is essentially non-fluorescent in water or MeOH.

### Product Description

SynaptoRed™ is a widely used red fluorescent cationic styryl dye for following synaptic activity by staining synaptic vesicles at the synapse or neuromuscular junctions. When used in combination with the green fluorescent dye SynaptoGreen™ (see Related Products), synapses or neuromuscular junctions can be imaged independently in two colors.

Nerve terminal 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™. SynaptoRed™ C2 is equivalent to FM@4-64.

Cationic styryl dyes stain synaptic vesicles in an activity-dependent fashion. In the presence of cells or tissue preparations, the dyes insert into the outer leaflet of the cell surface 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 membranes and thus have a higher on-rate and lower off-rate.

When using nerve terminal dyes, one frequent problem researchers encounter is background fluorescence due to non-specific 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 the 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. Biotium also offers AM dyes, which are aldehyde-fixable analogs of SynaptoGreen™ and SynaptoRed™ (see Related Products).

### References

1) Neuron 24(4), 809 (1999).

## Experimental 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.

**Note:** The addition of the sodium channel blocker tetrodotoxin (TTX) (see Related Products) 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 µM in 50 mM Tyrode solution (for example, 1 µL 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 0.5 µM TTX in Tyrode buffer for 1 minute at room temperature.
3. Wash for 5 minutes at room temperature with 0.5 µM TTX with ADVASEP-7 in Tyrode buffer. The typical concentration of ADVASEP-7 working solution is 1 mM (for example, 10 µL of 100 mM ADVASEP-7 per mL solution).
4. Rinse the coverslip twice with 0.5 µM TTX in Tyrode buffer.
5. Mount the coverslip in 0.5 µM TTX in Tyrode buffer and image.

**Note:** SynaptoRed™ C2 is not fixable. For fixable staining, we offer AM4-64, which is an aldehyde-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@1-84)
70048	SynaptoGreen™ C18 (equivalent to FM@3-25)
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
70053	HM1-43
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

Please visit our website at [www.biotium.com](http://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.

FM is a registered trademark of Thermo Fisher Scientific. Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.