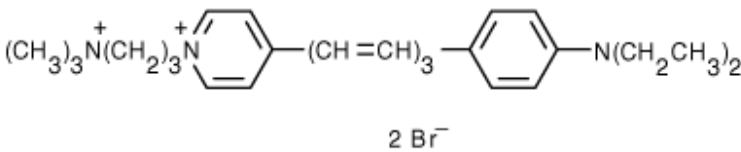


(also known as FM5-95, a trademark of Molecular Probes, Inc.)

## Instruction Manual

Catalog Number	PK-CA707-70019
Description	SynapseRed C2M (also known as FM5-95, a trademark of Molecular Probes, Inc.) is a red fluorescent probe used to follow synaptic vesicles. SynapseRed C2M is slightly more water soluble than SynapseRed C2 (also known as FM4-64, a trademark of Molecular Probes, Inc.).
Quantity	5 x 1 mg
Excitation / Emission Maxima	$\lambda_{exc}/\lambda_{em}$ (in MeOH) = 560/734 nm; Extinction coefficient (in MeOH) = 43,000
Molecular Structure	
Molecular Weight / Molecular Formula	565.43 Da; C <sub>27</sub> H <sub>39</sub> Br <sub>2</sub> N <sub>3</sub>
Purity	>99% (as determined by TLC)
Appearance / Formulation / Solubility	Dark purple solid; soluble in water or DMSO.
Storage & Stability	Store desiccated at -20°C. Protect from light, especially when in solution.
Applications	<p><u>Assay Protocol</u></p> <p>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 references for examples of protocols for staining brain slices and other cell types.</p> <ol style="list-style-type: none"> <li>1. Dilute nerve terminal dye to a final concentration of 4 μM in 50 mM Tyrode solution. Place the coverslip with your cells in this solution for 1 minute at room temperature. Use enough solution to completely submerge the cells.</li> <li>2. Transfer the coverslip to Tyrode + 0.5 μM tetrodotoxin (TTX, Cat.No. PK-CA707-00061) solution for 1 minute at room temperature.</li> <li>3. Wash the coverslip several times in Tyrode + TTX at room temperature. Note: to reduce background, 1 mM ADVASEP-7 (Cat.No. PK-CA707-70029) can be added to the wash solution. Alternatively, SCAS (Cat.No. PK-CA707-70037) can be used to quench background without repeated washes. Incubate the coverslip for 4 minutes at room temperature in Tyrode + TTX + 0.5 mM SCAS.</li> <li>4. Mount the coverslip in Tyrode + TTX and image. Note: for SynapseGreen dyes, 50 μM sulforhodamine 101 (Cat.No. PK-CA707-80101) can be included during mounting to quench extracellular fluorescence. Note: SynapseGreen and SynapseRed dyes are not fixable. The AM and HM dyes (see our website) are aldehyde-fixable nerve terminal dyes.</li> </ol>
References	<ol style="list-style-type: none"> <li>1) Science 297, 1525-1531 (2002)</li> <li>2) Cell 107, 605-616 (2001)</li> </ol>
Caution	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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