Product Information

NM4-64（AM4-64）

Catalog Number: N4072

Product Size: 1 mg

Application: Nerve terminal staining

Parameters

Appearance: Purple solid soluble in H_2O

Ex/Em (in MeOH): 543/- nm, (emission in MeOH is too weak to measure)

Ex/Em (in membranes): 510/750 nm

Molecular Formula: $C_{29}H_{45}N_4Cl_3$

Molecular Weight: 555.5

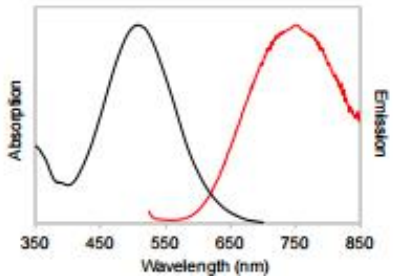

Figure 1. Excitation and emission spectra of NM4-64 in liposomes.

Storage

Store at -20℃ and protect from light. When stored as directed, product is stable for at least 12 months.

Description

Nerve terminal probes are a series of cationic styryl fluorescent dyes used to track synaptic activity at neuromuscular junctions or synapses. These dyes usually have a lipophilic tail (two carbon chains) and a highly hydrophilic head with cations. NM dyes have a similar structure to NerveRed or NerveGreen. In addition, they have an aldehyde-fixing amine on the positively charged head.

Cationic styryl dyes function by staining synaptic vesicles. When the dye is co-incubated with cells or tissues, the aqueous part of the dye is not fluorescent, and the lipophilic tail of the dye is inserted into the cell membrane and shows strong fluorescence. After the nerve is stimulated, it will undergo endocytosis. At this time, the dye is wrapped in the vesicle, and the intensity of the fluorescent signal indicates the number of newly formed vesicles. Conversely, during exocytosis, the dye is released from the vesicle along with the neurotransmitter, resulting in a decrease in the fluorescent signal. Therefore, changes in fluorescence intensity reflect the situation of endocytosis / exocytosis or synaptic activity. The rate of fluorescence increase (binding rate) during endocytosis and the rate of fluorescence decrease (dissociation rate) during exocytosis vary depending on the type of dye. Generally, dyes with longer lipophilic tails and more double bonds have higher affinity for the membrane and therefore have higher binding rates and lower off-rates.

NM4-64 dye can be used for fixed cell staining.

Protocol

The following is a nerve terminal staining protocol for neuronal cells cultured on coverslips.

Nerve terminal dyes can also be used to label endocytic vesicles of non-neuronal cell types. The plasma membrane can

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be selectively labeled at 4°C, and the labeled endocytosis can occur within 10min at room temperature or 37°C. Tyrode solution or other buffers can be used. The sodium ion channel blocker tetrodotoxin (TTX) can be optionally added to block the action potential and prevent the release of synaptic vesicles after staining. This protocol was optimized according to different experiments.

1. Dilute nerve ending dyes to a final concentration of 4 μM in 50 mM Tyrode solution. Place the coverslip containing the cells in this solution at room temperature for 1 min to completely submerge the cells.

2. Transfer coverslips to Tyrode + 0.5 μM tetrodotoxin (TTX) solution and incubate for 1 min at room temperature.

3. Wash the coverslips repeated at room temperature with Tyrode + 0.5 μM TTX solution.

4. Fixed. Coverslips were fixed with 4% paraformaldehyde for 20 min at room temperature.

5. Transfer the coverslips to pre-cooled 0.01% Triton X-100 in PBS and leave at 4 °C for 10 minutes.

6. Wash the cells three times with pre-cooled PBS for 1 min each.

7. Stain for 3h at 4 °C in 10% serum / PBS containing the primary antibody. The antibody concentration should be twice that of conventional immunofluorescence staining.

8. Wash the cells three times with pre-cooled PBS for 1 min each.

9. Stain for 40 min at 4 °C in 2% serum / PBS containing secondary antibodies. The antibody concentration should be the same as that of conventional immunofluorescence staining.

10. Wash the cells three times with pre-cooled PBS for 1 min each.

11. Observe with a fluorescence microscope.

Notes

1. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

2. For your safety and health, please wear lab coats and disposable gloves.

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