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ProductInformation

Nerve Terminal Stain Kits

Product Codes **NTS-I, NTS-II, NTS-V**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Nerve Terminal Stain Kits are used to detect and study synaptic vesicle recycling in neuronal synapses and neuromuscular junctions. Although each kit employs a different amphiphilic styryl pyridinium dye to visualize the vesicles, the tissue preparation, reagents required, and staining procedures are identical for the three kits. These dyes fluoresce intensely upon incorporation into plasma membranes whereas in aqueous environments the fluorescence is minimal.

Nerve Terminal Staining Kit-I (NTS-I) employs SynaptoGreen™ C4 (Product No. S6814)
Synonyms: N-(3-triethylammoniumpropyl)-4-(4-(dibutylamino)styryl)pyridinium dibromide); FM1-43
Molecular Formula: $C_{30}H_{49}Br_2N_3$
Molecular Weight: 611.55
λ_{ex} = 510 nm; λ_{em} = 625 nm in methanol
λ_{ex} = 480 nm; λ_{em} = 598 nm in membranes
Soluble in water, methanol, and DMSO

Nerve Terminal Staining Kit II (NTS-II) employs AM1-43 (Product No. A3848)
Molecular Formula: $C_{29}H_{49}Cl_3N_4$
Molecular Weight: 560.10
λ_{ex} = 510 nm; λ_{em} = 625 nm in methanol
λ_{ex} = 480 nm; λ_{em} = 598 nm in membranes
Soluble in water, methanol and DMSO
AM1-43 has fluorescence properties similar to those of SynaptoGreen C4, but its additional amine group allows it to be fixed by aldehyde fixing agents. Thus, it is suitable for fluorescence microscopy applications.

Nerve Terminal Staining Kit V (NTS-V) employs SynaptoRed™ C2 (Product No. S6689)
Synonym: N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide
Molecular Formula: $C_{30}H_{45}Br_2N_3$
Molecular Weight: 607.51
λ_{ex} = 543 nm; λ_{em} = too weak to detect in methanol
λ_{ex} = 515 nm; λ_{em} = 640 nm in membranes
Soluble in water, methanol and DMSO

All three kits also employ the sulfonated β-cyclodextrin Advasep-7 (Product No. A 3723)
Molecular Formula: $C_{42}H_{70-n}O_{35}(C_4H_6SO_3Na)_n$ where, on average, n = 6.5
Molecular Weight: approximately 2163
Soluble in water

When neurons are actively releasing neurotransmitters, these dyes are incorporated into recycled vesicles at the presynaptic terminal. The dye-containing vesicles are not susceptible to subsequent wash steps that remove the excess dye from the outer leaflet of the plasma membrane. Thus only active presynaptic terminals are labeled with the dye. Advasep-7 further reduces background by encapsulating the probes, preventing stain from accumulating interstitially. Advasep-7 is applied as a rinse at various stages of the staining process.

Components and Reagents

Product Code	Item	Quantity
NTS-I	Nerve Terminal Stain Kit-I	
S6814	SynaptoGreen™ C4	5x1 mg
A3723	Advasep-7	500 mg
NTS-II	Nerve Terminal Stain Kit-II	
A3848	AM1-43	1x1mg
A3723	Advasep-7	100 mg
NTS-V	Nerve Terminal Stain Kit-V	
S6689	SynaptoRed™ C2	5x1mg
A3723	Advasep-7	500 mg

Preparation Instructions
Note: All solutions are pH 7.4 and filter sterilized.

Dyes: All dyes are reconstituted in DMSO (Product No. D2650) at a stock concentration of 10 mM and stored in the dark at 4 °C. Working dilutions (1-15 µM in Solution D, see below) are made on the day of use and are stored in the dark at 4 °C prior to use. Working dilutions are stable for at least one week under these storage conditions.

ADVASEP-7 is reconstituted in water at a concentration of 1 mM.

Materials Required But Not Provided

Solution A	**Solution B**
Wash Buffer	**Wash Buffer plus Ca^{2+}**
119 mM NaCl (S5886)	119 mM NaCl (S5886)
2.5 mM KCl (P5405)	2.5 mM KCl (P5405)
4 mM $MgCl_2$ (M2393)	4 mM $MgCl_2$ (M2393)
30 mM Glucose (G7021)	30 mM Glucose (G7021)
25 mM HEPES (H4034)	25 mM HEPES (H4034)
	2 mM $CaCl_2$ (C7902)
Usage: To remove dyes from non-synaptic regions of plasma membrane after loading or unloading	Usage: To load the coverslip into the imaging chamber and dye unloading

Solution C	**Solution D**
Stimulation Solution	**Dye loading Solution**
60-90 mM KCl (P5405)	60-90 mM KCl (P5405)
29 mM NaCl (S5886)	29 mM NaCl (S5886)
2 mM $CaCl_2$ (C7902)	2 mM $CaCl_2$ (C7902)
2 mM $MgCl_2$ (M2393)	2 mM $MgCl_2$ (M2393)
25 mM HEPES (H4034)	25 mM HEPES (H4034)
30 mM Glucose (G7021)	30 mM Glucose (G7021)
	1-15 µM Dye
Usage: To cause presynaptic release, vesicle fusion, and dye unloading	Usage: To cause presynaptic release, vesicle fusion, and dye incorporation

Precautions and Disclaimer
For Research Use Only.

Procedure
Sample Protocol used with dissociated primary hippocampal neurons in Banker culture:
The following is suggested as a general protocol and may require optimization for use with other tissue types, conditions, and/or applications.

Growth of Cells
Use the growth conditions (medium, temperature, O_2/CO_2 concentration, etc.) recommended for the cell line being studied. The cells should be grown to 21-28 DIV (days *in vitro*) on an appropriate platform, e.g. slide, coverslip, or chamber slide.

Mount coverslips in the open, unheated imaging chamber following introduction of dye loading solution as described below.

Staining Procedure
Setup

1. Prepare the perfusion apparatus by purging air from the tubing with Solution A, Set the flow rate to 2-3 ml/min. Connect a vacuum line to remove buffer from imaging chamber during perfusion.
2. Load the live hippocampal neurons into the unheated open imaging chamber of the perfusion apparatus with 500 µl of Solution B.
3. Gently aspirate the excess Solution B using a Pasteur pipette. DO NOT remove all of the media as this will dry the neurons.

Dye Loading (2 x 20 sec. incubations)

4. Add approximately 250 µl of Solution D to the chamber and begin timing first 20 second incubation period.
5. Following incubation, remove the spent Solution D from the chamber with a Pasteur pipette and add a fresh 250 µl volume of Solution D.
6. Incubate for approximately 20 seconds.
7. Wash the cells by perfusion with Solution A at a rate of 2-3 ml/min for up to 10 minutes.

Advasep-7 Wash

8. Gently aspirate the excess Solution A from the washed cells using a Pasteur pipette. DO NOT remove all of the media as this will dry the neurons.
9. Add 250 µl of 1mM ADVASEP-7.
10. Incubate for 1-2 min
11. Wash the cells by perfusion with Solution A at a rate of 2-3 ml/min. Wash for 10-20 minutes or until the background is sufficiently removed.

Dye Unloading (2 x 20 sec. incubations)

12. Gently aspirate the excess Solution A from the washed cells using a Pasteur pipette. DO NOT remove all of the media as this will dry the neurons.
13. Add 250 µl of Solution C. Begin timing first 20 second incubation period.
14. Following incubation, remove the spent Solution C from the chamber with a Pasteur pipette. Add a fresh 250 µl volume of Solution C.
15. Incubate for approximately 20 seconds.
16. Wash the cells by perfusion with Solution A at a rate of 2-3 ml/min for 10-20 minutes.

Note: if dyes do not unload sufficiently, try reducing exposure time to Solution A during the perfusion washes following the dye loading and Advasep-7 loading steps.

References

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LCM/LY 04/02