Sema-3A & NRP1/Plexin A1 Receptor
Immunocytochemistry Kit

Cat. # SK7750

Cat. #	Description	Product Type	Size	Applications	Species Reactivity	ICC Dilution
NP2111	Neuropilin-1 (a1 CUB Domain)	Rabbit pAb	50 µl	WB, E, ICC, IHC	Hu, Rt, Ms	1:200
PP1301	Plexin A1 (Sema Domain)	Rabbit pAb	50 µl	WB, E, IP, ICC, IHC	Hu, Rt, Ms	1:100
SP1221	Semaphorin-3A (Central region)	Rabbit pAb	50 µl	WB, E, ICC, IHC	Hu, Rt, Ms	1:100
RS3261	Anti-Rabbit Ig:DyLight® 488	Goat pAb	100 µl	ICC, IHC	Rb	1:200
RS3271	Anti-Rabbit Ig:DyLight® 594	Goat pAb	100 µl	ICC, IHC	Rb	1:200

Applications: WB = Western blot, E = ELISA, ICC = Immunocytochemistry, IP = Immunoprecipitation, IHC = Immunohistochemistry, FC = Flow Cytometry
Species: H = Human, R = Rat, M = Mouse, C = Chicken, F = Fish, Fr = Frog, Rb = Rabbit

Kit Summary

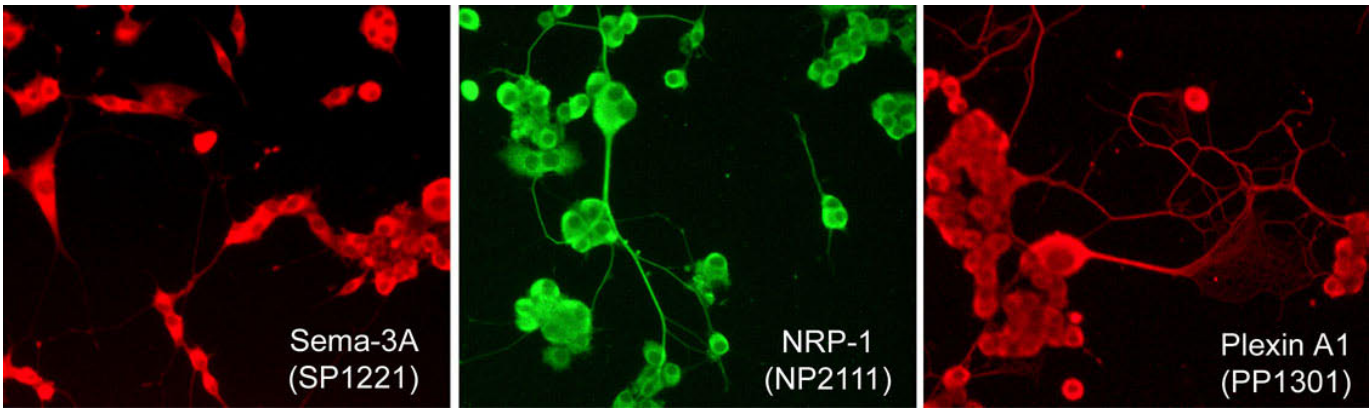
The Sema-3A & NRP1/Plexin A1 receptor kit can be used for immuncytochemical labeling of Sema-3A relative to its receptors, NRP1 and Plexin A1. The kit also includes secondary reagents conjugated to DyLight® 488 or DyLight® 594 for detection of primary antibodies using green (493Ex/518Em) or red (593Ex/618Em) filter sets.

Buffers and Storage

Rabbit polyclonal antibodies and secondary reagents are supplied in phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Store at –20°C. Stable for 1 year.

Background

One family of inhibitory axon guidance molecules is the semaphorins. The semaphorins include secreted, transmembrane, and GPI-anchored extracellular molecules that are involved in regulating axon guidance by inhibiting axons from growing toward incorrect targets. Semaphorin 3A (Sema3A) may play a particularly interesting role in limiting axon regeneration since it is expressed in meningeal fibroblasts that invade the injured spinal cord and surround the glial scar. In addition, the Sema3A co-receptors, Neuropilin-1 and Plexin-A1, are expressed on axons that regenerate up to the injured region, but do not cross this Sema3A-containing region. Thus, Sema3A and its co-receptors may have important roles in regulating axon guidance during neuronal development and after neuronal injury.



Immunocytochemical labeling of Sema-3A and its receptors in aldehyde-fixed and NP-40-permeabilized NGF-differentiated PC12 cells. The cells were labeled with rabbit polyclonal anti-Sema-3A (C-terminal) (SP1221), anti-NRP1 (NP221), and anti-Plexin A1 (PP1301). The antibodies were detected using appropriate secondary antibody conjugated to either DyLight® 488 or DyLight® 594.

www.ecmbiosciences.com
telephone: 859-879-2075
toll-free: 1-800-859-8202
info@ecmbiosciences.com

ECM Biosciences

Rev 1/22/2014

Sema-3A & NRP1/Plexin A1 Receptor
Immunocytochemistry Kit

Cat. # SK7750

Adherent Cell Fixation

1. Remove cell growth medium from culture plate containing cells, and rinse cells once with Hank's buffered saline solution (HBSS) or other rinse buffer acceptable for your cell type.

2. Fix cells with 4% Paraformaldehyde/0.2% NP-40 in HBSS for 30 minutes at room temperature.

   Note: Some antibodies work better for immunocytochemistry using one of the following methods:
   A. Methanol/Acetone fixation: Fix and permeabilize in 1:1 Methanol/Acetone at -20°C for 10 min.
   B. Aldehyde/Acetone fixation: Fix cells with 4% Paraformaldehyde in HBSS for 30 minutes at room temperature, then permeabilize for 15 min. with 100% Acetone for at -20°C.

3. Remove fixation solution and rinse cells two times with phosphate buffered saline solution (PBS).

4. Block non-specific binding sites with 1% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature.

   Note: Normal animal serum (e.g. horse, goat) that matches the species of the secondary antibody can be substituted for BSA, if non-specific labeling occurs with certain secondary reagents.

Primary Antibody Labeling

5. Make primary antibody dilutions in 1% BSA in PBS, using the recommended dilution described in the table above. For some cell types, the optimal antibody dilution may need to be empirically determined. Titrations of 1:50 to 1:500 can be useful to determine the optimal dilution for each primary antibody.

6. Remove the blocking solution from step #4, then add primary antibody dilutions and incubate for 1-2 hours at room temperature.

7. After primary antibody probing, rinse cells three times with PBS.

Secondary Antibody Labeling

8. Make secondary dilutions in 1% BSA (or normal serum) in PBS.

9. Suggested dilutions for secondary antibodies used at ECM Biosciences:

RS3261 Goat anti-Rabbit Ig:DyLight® 488	(Green; Abs./Em. = 493/518)	1:200
MS3011 Goat anti-Mouse Ig:DyLight® 488	(Green; Abs./Em. = 493/518)	1:200
RS3271 Goat anti-Rabbit Ig:DyLight® 594	(Red; Abs./Em. = 593/618)	1:200
MS3031 Goat anti-Mouse Ig:DyLight® 594	(Red; Abs./Em. = 593/618)	1:200

10. Add secondary antibody to cells for 30 minutes at room temperature.
    Note: Fluorescent secondary antibody labeling should be performed in the dark.

11. For long-term storage (months at 4°C), remove PBS and add SlowFade Gold (Invitrogen) to the cells and seal the slides or plates.

DyLight® is a trademark of Thermo Scientific, Inc. and its subsidiaries.

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

www.ecmbiosciences.com
telephone: 859-879-2075
toll-free: 1-800-859-8202
info@ecmbiosciences.com

ECM Biosciences

Rev 1/22/2014