Store at -20°C

#8572

Neuronal Marker IF Antibody Sampler Kit

☑ 1 Kit
(5 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype	IF-F Dilution
GFAP (D1F4Q) XP® Rabbit mAb	12389	20 µl	50 kDa	Rabbit IgG	1:200
CNPase (D83E10) XP® Rabbit mAb	5664	20 µl	47 kDa	Rabbit IgG	1:100
β3-Tubulin (D71G9) XP® Rabbit mAb	5568	20 µl	55 kDa	Rabbit IgG	1:200
Nestin (Rat-401) Mouse mAb	4760	20 µl	207 kDa	Mouse IgG1	1:300
Neurofilament-L (C28E10) Rabbit mAb	2837	20 µl	70 kDa	Rabbit IgG	1:100

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Please visit www.cellsignal.com for validation data and a complete listing of recommended dilutions and companion products.

Description: The Neuronal Marker IF Antibody Sampler Kit provides an economical means for labeling neuronal structures by immunofluorescence (IF-F).

Background: The antibodies in this kit serve as neuronal markers to determine protein localization in neurons. The cytoskeleton consists of three types of cytosolic fibers: microfilaments (actin filaments), intermediate filaments, and microtubules. Neurofilaments are the major intermediate filaments found in neurons and consist of light (NFL), medium (NFM), and heavy (NFH) subunits (1). Nestin is an intermediate filament family member protein that is structurally related to the neurofilament proteins (2). Globular tubulin subunits comprise the microtubule building block, with α/β-tubulin heterodimers forming the tubulin subunit common to all eukaryotic cells (3). High CNPase expression is seen in oligodendrocytes and Schwann cells as CNPase accounts for roughly 4% of the total myelin protein in the central nervous system (4). CNPase binds to tubulin heterodimers and plays a role in tubulin polymerization and oligodendrocyte process outgrowth (5). GFAP filaments are characteristic of differentiated and mature brain astrocytes. Thus, GFAP is commonly used by investigators as a marker for intracranial and intraspinal tumors arising from astrocytes (6).

Specificity/Sensitivity: Each antibody in the Neuronal Marker IF Antibody Sampler Kit has been validated for IF-F, recognizes only its specific target, and does not cross-react with other family members. CNPase (D83E10) XP® Rabbit mAb recognizes endogenous levels of total CNPase protein. GFAP (D1F4Q) XP® Rabbit mAb detects endogenous levels of total GFAP protein. Nestin (Rat-401) Mouse mAb detects endogenous levels of nestin protein. Neurofilament-L (C28E10) Rabbit mAb detects endogenous levels of total Neurofilament-L protein. β3-Tubulin (D71G9) XP® Rabbit mAb detects endogenous levels of total β3-tubulin protein. This antibody does not cross-react with tubulin isoforms expressed in non-neuronal cells. This clone is similar to TUJ1. Expression of these proteins may vary in different cells and tissues This antibody does not cross-react with tubulin isoforms expressed in non-neuronal cells. This clone is similar to TUJ1. Expression of these proteins may vary in different cells and tissues.

Source/Purification: Rabbit monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp395 of human GFAP protein, or the carboxy terminus of human b3-tubulin protein, or residues surrounding Val81 of human CNPase protein, or Glu450 of human Neurofilament-L protein.

Background References:

(1) Al-Chalabi, A. and Miller, C.C. (2003) *Bioessays* 25, 346-55.

(2) Michalczyk, K. and Ziman, M. (2005) *Histol Histopathol* 20, 665-71.

(3) Westermann, S. and Weber, K. (2003) *Nat Rev Mol Cell Biol* 4, 938-47.

(4) Kozlov, G. et al. (2003) *J Biol Chem* 278, 46021-8.

(5) Lee, J. et al. (2005) *J Cell Biol* 170, 661-73.

(6) Goebel, H.H. et al. (1987) *Acta Histochem Suppl* 34, 81-93.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#8572

Immunofluorescence Protocol

***IMPORTANT:** Please refer to the **APPLICATIONS** section on the front page of the data sheet to determine **IF THIS PRODUCT** is validated and approved for the specific protocol you will be using.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. **10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O. Adjust pH to 7.4.
2. Formaldehyde, 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark, dilute in PBS for use.
3. Xylene
4. Ethanol, anhydrous denatured, histological grade, 100% and 95%
5. Distilled water (dH_2O)
6. **Blocking Buffer:** To prepare 25 mL, add 2.5 mL 10X PBS, 1.25 mL normal serum from the same species as the secondary antibody (eg. normal goat serum, normal donkey serum) and 21.25 mL dH_2O and mix well. While stirring, add 75 µL Triton X-100 (100%).
7. **Antibody Dilution Buffer:** To prepare 40 mL, add 4 mL 10X PBS to 36 mL dH_2O, mix. Add 0.4 g BSA and mix well. While stirring, add 120 µL Triton X-100 (100%).
8. **10 mM Sodium Citrate Buffer:** To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate ($C_6H_5Na_3O7 \bullet 2H_2O$) to 1 L dH_2O. Adjust pH to 6.0.
9. **1X PBS, high salt (0.4M) (high salt PBS):** To prepare 1L, add 100 ml 10X PBS to 900 ml dH20. Add 23.38 g NaCl and mix.
10. Fluorochrome-conjugated secondary antibody

NOTE: When using any primary or fluorochrome-conjugated secondary antibody for the first time, titrate the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

11. Prolong® Gold Antifade Reagent (Invitrogen, Eugene, OR, Cat# P36930)

B Specimen Preparation

I. Cultured Cell Lines (IF-IC)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-IC).**

NOTE: Cells should be grown, treated, fixed, and stained directly in multiwell plates, chamber slides, or on coverslips.

1. Rinse cells briefly in PBS.
2. Aspirate PBS, cover cells to a depth of 2-3 mm with 2-4% formaldehyde in PBS.

NOTE: Formaldehyde is toxic, use only in fume hood.

3. Allow cells to fix for 15 minutes at room temperature.
4. Aspirate fixative, rinse three times in PBS for 5 minutes each.
5. **Methanol Permeabilization Step (if required, please refer to front page):** After formaldehyde fixation, cover cells with ice-cold 100% methanol (use enough to cover cells completely to a depth of 3-5 mm, DO NOT LET CELLS DRY), incubate cells in methanol for 10 minutes at –20°C, rinse in PBS for 5 minutes.
6. Proceed with Immunostaining section C.

II. Paraffin Sections (IF-P)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-P).**

Deparaffinization/Rehydration:

1. Incubate sections in three washes of xylene for 5 minutes each.
2. Incubate sections in two washes of 100% ethanol for 10 minutes each.
3. Incubate sections in two washes of 95% ethanol for 10 minutes each.
4. Rinse sections twice in dH_2O for 5 minutes each.

Antigen Unmasking:

1. Place slides in room temperature 10 mM sodium citrate buffer pH 6.0.
2. Bring slides to boiling in sodium citrate buffer using water bath or microwave, then maintain at 95-99°C for 10 minutes.
3. Cool slides for 30 minutes on bench top.
4. Rinse sections in dH_2O three times for 5 minutes each.
5. Rinse sections in PBS for 5 minutes.
6. Proceed with Immunostaining section C.

III. Frozen/Cryostat Sections (IF-F)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-F).**

NOTE: Fresh frozen/unfixed sections should be fixed immediately in 2-4% formaldehyde as follows to preserve signaling epitopes.

1. Cover sections with 2-4% formaldehyde in PBS

NOTE: Formaldehyde is toxic, use only in fume hood.

2. Allow sections to fix for 15 minutes at room temperature.
3. Rinse slides three times in PBS for 5 minutes each.

C Immunostaining

NOTE: All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

1. Block specimen in Blocking Buffer for 60 minutes.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in Antibody Dilution Buffer.
3. Aspirate blocking solution, apply diluted primary antibody.

NOTE: For double-labeling, prepare a cocktail of the primary antibodies at their appropriate dilution in Antibody Dilution Buffer.

4. Incubate overnight at 4°C.
5. Rinse three times in PBS for 5 minutes each.

OPTION: To decrease background stain, rinse in high salt PBS for two minutes between second and third PBS rinses. Be aware, this may reduce specific staining of some antibodies.

NOTE: If using primary antibodies directly conjugated with Alexa Fluor® fluorochromes, then skip to step C8.

6. Incubate specimen in fluorochrome-conjugated secondary antibody diluted in Antibody Dilution Buffer for 1-2 hours at room temperature in dark.

NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated secondary antibodies at their appropriate dilutions in Antibody Dilution Buffer.

7. Rinse in PBS/high salt PBS as in step 5.
8. Coverslip slides with Prolong® Gold Antifade Reagent or apply just enough to cover cells in multiwell plate.
9. Seal slides by painting around edges of coverslips with nail polish.
10. For best results examine specimens immediately using appropriate excitation wavelength. For long term storage, store slides flat at 4°C protected from light.