#12823 Store at -20°C

Plexin Isoform Antibody Sampler Kit

☑ 1 Kit
(4 x 40 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
Plexin A1 Antibody	3813	40 µl	211 kDa	Rabbit IgG
Plexin A2 (D42B5) Rabbit mAb	5658	40 µl	212 kDa	Rabbit IgG
Plexin A3 (D2G12) Rabbit mAb	5512	40 µl	207 kDa	Rabbit IgG
Plexin A4 (C5D1) Rabbit mAb	3816	40 µl	212 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Description: The Plexin Isoform Antibody Sampler Kit provides an economical means of evaluating Plexin A1, A2, A3 and A4 protein expression. The kit contains enough primary antibody to perform four western miniblot experiments per primary antibody.

Background: Class 3 secreted semaphorin (Sema3A) is a chemorepellent that acts upon a wide variety of axons. As such, it induces a dramatic redistribution and depolymerization of actin filaments that results in growth cone collapse. Plexins are single membrane-spanning signaling proteins encompassing Plexin A1, A2, A3, and A4. Plexins form a complex with neuropilin-1 and -2 and the cell adhesion protein L1 to form a functional semaphorin receptor (1,2). The GTPase Rnd1 binds to the cytoplasmic domain of Plexin A1 to trigger cytoskeletal collapse. In contrast, the GTPase RhoD blocks Rnd1-mediated Plexin A1 activation and repulsion of sympathetic axons by Sema3A (3). Sema6A is a ligand for Plexin A2. Both Sema6A and Plexin A2 knockout mice have a granule cell migration defect, where cells remain in the molecular layer. Furthermore, Plexin A2 also controls nucleus-centrosome coupling that modulates cell migration (4). Both Plexin A3 and A4 mediate the responses to class 3 semaphorins in sensory and sympathetic neurons. In particular, Plexin A4 is responsible for signaling of Sema 3A via neuropilin-1, while Plexin A3 is responsible for signaling of Sema 3F via neuropilin-2 (5).

Specificity/Sensitivity: Plexin A1 Antibody detects endogenous levels of total Plexin A1 protein. Plexin A2 (D42B5) Rabbit mAb detects endogenous levels of total Plexin A2 protein and may cross-react with unidentified proteins at various molecular weights. Plexin A3 (D2G12) Rabbit mAb detects endogenous levels of total Plexin A3 protein and may cross-react with other family members. Plexin A4 (C5D1) Rabbit mAb detects endogenous levels of total Plexin A4 protein.

Source/Purification: Polyclonal antibody is produced by immunizing animals with a synthetic peptide surrounding Gln1875 of human Plexin A1 protein and purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly185 of human Plexin A2 protein, or Val440 of human Plexin A3 protein, or with a GST-fusion protein corresponding to residues from the human sequence of Plexin A4 protein.

Background References:

(1) Pasterkamp, R.J. and Kolodkin, A.L. (2003) *Curr Opin Neurobiol* 13, 79-89.

(2) Takahashi, T. and Strittmatter, S.M. (2001) *Neuron* 29, 429-39.

(3) Zanata, S.M. et al. (2002) *J Neurosci* 22, 471-7.

(4) Renaud, J. et al. (2008) *Nat Neurosci* 11, 440-9.

(5) Yaron, A. et al. (2005) *Neuron* 45, 513-23.

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.*

Recommended Antibody Dilutions:

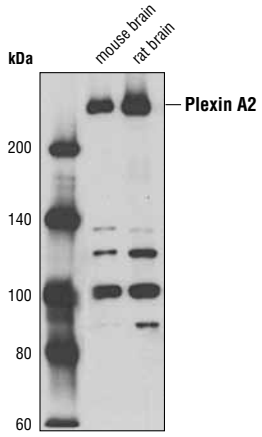
Western blotting	1:1000

Please visit www.cellsignal.com for a complete listing of recommended companion products.

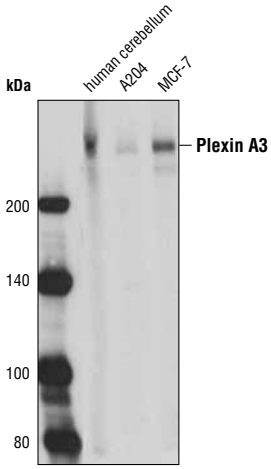
U.S. Patent No. 5,675,063

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

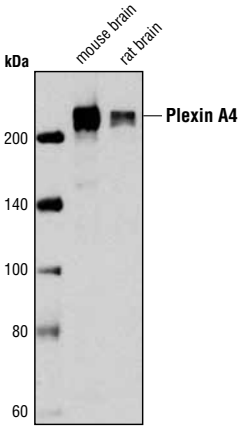
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted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



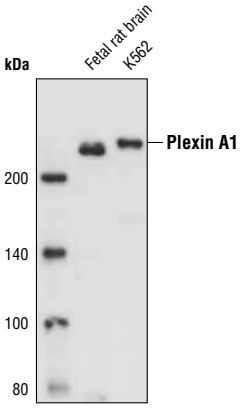
Western blot analysis of extracts from mouse and rat brain tissue using ***Plexin A2 (D42B5) Rabbit mAb #5658****.*



Western blot analysis of extracts from human cerebellum, A204 and MCF-7 cells using ***Plexin A3 (D2G12) Rabbit mAb #5512****.*



Western blot analysis of extracts from mouse and rat brain tissues using ***Plexin A4 (C5D1) Rabbit mAb #3816****.*



Western blot analysis of extracts from fetal rat brain and K-562 cells using ***Plexin A1 Antibody #3813****.*

#12823

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

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

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween®20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween®20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween®20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween®20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.