

Neuronal Scaffold Proteins Antibody Sampler Kit

✓ 1 Kit
(4 x 40 µl)



Orders ■ 877-616-CELL (2355)
orders@cellsignaling.com
Support ■ 877-678-TECH (8324)
info@cellsignaling.com
Web ■ www.cellsignaling.com

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
PSD95 (D27E11) XP® Rabbit mAb	3450	40 µl	95 kDa	Rabbit IgG
Homer1 Antibody	8231	40 µl	46 kDa	Rabbit IgG
SHANK2 Antibody	12218	40 µl	165 kDa	Rabbit IgG
Spinophilin (E1E7R) Rabbit mAb	14136	40 µl	130 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Description: The Neuronal Scaffold Proteins Antibody Sampler Kit provides an economical means of evaluating four major scaffolding proteins. The kit includes enough primary antibody to perform four western blot experiments.

Background: Scaffold proteins are composed of protein-interaction domains that tether multiple components of a signaling pathway to form signal transduction complexes. This organization of signaling molecules can help enhance signaling specificity and speed. Scaffold proteins are central components in neuronal synapses, where dynamic trafficking of synaptic proteins occurs. Mutations in scaffold proteins could have significant impact on synaptic structure and function. Postsynaptic density protein 95 (PSD95) is a member of the membrane-associated guanylate kinase (MAGUK) family of proteins and a scaffolding protein involved in the assembly and function of the postsynaptic density complex (1,2). SHANK proteins act as scaffolds at the neuronal postsynaptic density (PSD), where they play a critical role in PSD assembly of excitatory synapses during development (3,4). While recruitment of SHANK proteins to the synapse is independent of their interaction with Homer (5), proper synaptic targeting of SHANK1 is mediated by interactions between its PDZ domain and PSD proteins (6). Homer proteins (1-3) are scaffolds, composed of an EVH protein-binding domain, a coiled-coil domain and a leucine zipper domain. The EVH domain is a protein-protein binding module that binds to the proline-rich motifs of G protein-coupled receptors (GPCRs), inositol 1,4,5-triphosphate (IP3) receptors (IP3Rs), ryanodine receptors, and TRP channels (7,8). The coiled-coil and the leucine zipper domains cause multimerization of Homers and assemble signaling proteins complexes. Spinophilin is a protein phosphatase 1 regulatory protein that interacts with a large number of proteins, including ion channel components and G protein-coupled receptors (GPCRs). Spinophilin also interacts with actin filaments; phosphorylation of spinophilin at Ser94 and Ser177 disrupts this interaction (9,10).

Specificity/Sensitivity: PSD95 (D27E11) XP® Rabbit mAb detects endogenous levels of total PSD95 protein. SHANK2 Antibody recognizes endogenous levels of total SHANK2 protein. Homer1 Antibody detects endogenous levels of total Homer1 protein. This antibody may also detect isoform Homer1c. Spinophilin (E1E7R) Rabbit mAb recognizes endogenous levels of total spinophilin protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Val537 of human SHANK2 protein or residues surrounding Glu130 of human Homer1 protein. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly99 of human PSD95 or residues surrounding Pro298 of human spinophilin protein.

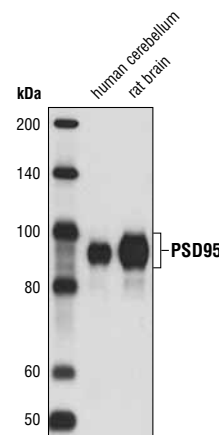
Background References:

- (1) Cao, J. et al. (2005) *J Cell Biol* 168, 117-26.
- (2) Chetkovich, D.M. et al. (2002) *J Neurosci* 22, 6415-25.
- (3) Grabrucker, A.M. et al. (2011) *Trends Cell Biol* 21, 594-603.
- (4) Boeckers, T.M. et al. (1999) *J Neurosci* 19, 6506-18.
- (5) Boeckers, T.M. et al. (2005) *J Neurochem* 92, 519-24.
- (6) Sala, C. et al. (2001) *Neuron* 31, 115-30.
- (7) Fagni, L. et al. (2002) *Sci STKE* 2002, re8.
- (8) Yuan, J.P. et al. (2003) *Cell* 114, 777-89.
- (9) Sarrouilhe, D. et al. (2006) *Biochimie* 88, 1099-113.
- (10) Hsieh-Wilson, L.C. et al. (2003) *J Biol Chem* 278, 1186-94.

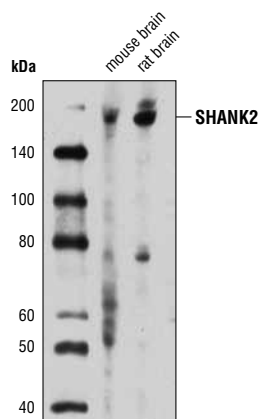
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

Recommended Antibody Dilutions:
Western blotting 1:1000

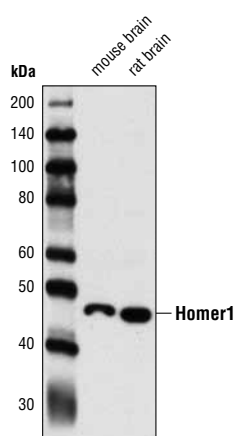
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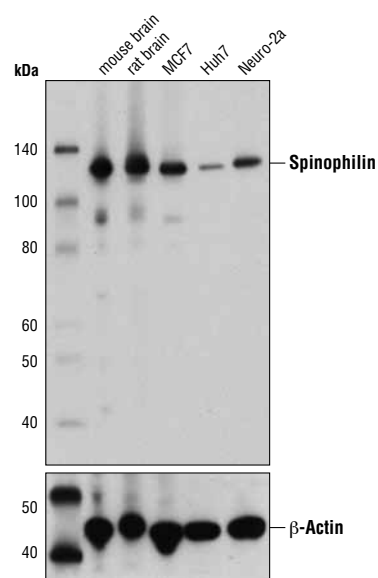
Western blot analysis of extracts from human cerebellum and rat brain tissue using **PSD95 (D27E11) XP® Rabbit mAb #3450**.



Western blot analysis of extracts from mouse and rat brain tissue using **SHANK2 Antibody #12218**.



Western blot analysis of extracts from mouse and rat brain tissue using **Homer1 Antibody #8231**.



Western blot analysis of extracts from various tissues and cell lines using **Spinophilin (E1E7R) Rabbit mAb #14136** (upper) and **β -Actin (D6A8) Rabbit mAb #8457** (lower).

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723)
Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
- Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

- Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 min each with 15 ml of TBST.
- Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.
NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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