#9967 Store at -20°C

Actin Reorganization Antibody Sampler Kit

☑ 1 Kit
(7 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
Phospho-Cofilin (Ser3) (77G2) Rabbit mAb	3313	20 µl	19 kDa	Rabbit IgG
Cofilin (D3F9) XP® Rabbit mAb	5175	20 µl	19 kDa	Rabbit IgG
Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) (48G2) Rabbit mAb	3726	20 µl	75 kDa Moesin. 80 kDa Ezrin, Radixin	Rabbit IgG
Ezrin/Radixin/Moesin Antibody	3142	20 µl	75 kDa Moesin. 80 kDa Ezrin, Radixin	Rabbit IgG
Phospho-VASP (Ser157) Antibody	3111	20 µl	51 kDa	Rabbit IgG
Phospho-VASP (Ser239) Antibody	3114	20 µl	48, 51 kDa	Rabbit IgG
VASP (9A2) Rabbit mAb	3132	20 µl	46, 50 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.

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

Recommended Antibody Dilutions:

Western blotting	1:1000

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

Description: The Actin Reorganization Antibody Sampler Kit contains reagents to examine proteins that help regulate the dynamic actin cytoskeleton. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Ubiquitous actin protein comprises the major structural component of the eukaryotic cytoskeleton. The formation and continual reorganization of the actin cytoskeleton is a key step in many biological processes, including cell motility, cytokinesis, endocytosis, embryonic development, tissue regeneration and the stress response (1). The small protein cofilin is one of a conserved family of actin-binding proteins that promote actin filament regeneration by severing preexisting filaments (2). Phosphorylation of cofilin at Ser3 by LIMK or TESK inhibits cofilin severing activity (3-5). Ezrin, radixin, and moesin (ERM) proteins function as linker proteins and signal transducers between the plasma membrane and actin cytoskeleton. These proteins are involved in cell adhesion, membrane ruffling, and microvilli formation (6,7). Interactive cytosolic ERM proteins exist as monomers or dimers that form both intra- and intermolecular associations through their amino- and carboxy-terminal domains (8). Phosphorylation at carboxy-terminal threonine residues (Thr567 of ezrin, radixin at Thr564 and Thr558 of moesin) may alter protein conformation and disrupt these protein associations and result in ERM protein activation (9,10). Vasodilator-stimulated phosphoprotein (VASP) is an adaptor protein that links the cytoskeleton with signal transduction pathways to act in fibroblast migration, platelet activation and axon guidance (11,12). Three phosphorylation sites (Ser157, Ser239, and Thr278) have been identified, with phosphorylation of Ser239 by PKG serving as a marker for nitric oxide and cGMP signaling (13). VASP Ser157 can act as a substrate for both PKA and PKC (14,15). Active VASP appears to promote actin polymerization by restricting actin filament capping, with PKA phosphorylation inhibiting this anti-capping activity (16).

Specificity/Sensitivity: All activation state antibodies only detect their target proteins when modified at the indicated site. Cofilin (D3F9) XP® Rabbit mAb detects endogenous levels of total cofilin protein. VASP (9A2) Rabbit mAb detects endogenous levels of total VASP protein. Neither of the Ezrin/Radixin/Moesin antibodies cross-react with related phosphoproteins such as merlin or band 4.1.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to central residues of human cofilin1 and residues near the carboxy terminus of human and mouse VASP. Monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser3 of human cofilin and Thr567 of human ezrin. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to Thr567 of human ezrin. Activation state polyclonal antibodies are produced by immunizing rabbits with synthetic phosphopeptides corresponding to residues surrounding Ser157 and Ser239 of human VASP. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

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(2) Condeelis, J. (2001) *Trends Cell Biol.* 11, 288–293.
(3) Arber, S. et al. (1998) *Nature* 393, 805-809.
(4) Yang, N. et al. (1998) *Nature* 393, 809-812.
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(6) Louvet-Vallée, S. (2000) *Biol. Cell* 92, 305-316.
(7) Ivetic, A. and Ridley, A.J. (2004) *Immunology* 112, 165–176.
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(10) Tran Quang, C. et al. (2000) *EMBO J.* 19, 4565–4576.
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(12) Machesky, L.M. (2000) *Cell* 101, 685–688.
(13) Ibarra-Alvarado, C. et al. (2002) *Mol. Pharmacol.* 61, 312–319.
(14) Smolenski, A. et al. (1998) *J. Biol. Chem.* 273, 20029–20035.
(15) Chitaley, K. et al. (2004) *FEBS Lett.* 556, 211–215.
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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 **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **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 dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **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 dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **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.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **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.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. 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.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

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

II. Primary Antibody Incubation

1. 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.
2. Wash three times for 5 min each with 15 ml of TBST.
3. 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.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. 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.
2. 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.