#8354 Store at -20°C

Cofilin Activation Antibody Sampler Kit

☑ 1 Kit
(7 x 20 µl)

Orders ■ 877-616-CELL (2355)
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Support ■ 877-678-TECH (8324)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Cofilin (D3F9) XP® Rabbit mAb	5175	20 µl	19 kDa	Rabbit IgG
Phospho-Cofilin (Ser3) (77G2) Rabbit mAb	3313	20 µl	19 kDa	Rabbit IgG
LIMK2 (8C11) Rabbit mAb	3845	20 µl	70 kDa	Rabbit IgG
TESK1 (D49D4) Rabbit mAb	4655	20 µl	68 kDa	Rabbit IgG
ROCK1 (C8F7) Rabbit mAb	4035	20 µl	160 kDa	Rabbit IgG
Chronophin/PDXP (C85E3) Rabbit mAb	4686	20 µl	31 kDa	Rabbit IgG
SSH1 (E1K3W) Rabbit mAb	13578	20 µl	140 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, 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 validation data and a complete listing of recommended companion products.

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

Description: The Cofilin Activation Antibody Sampler Kit provides an economical means to evaluate the presence and status of cofilin activation. The kit contains enough primary antibody to perform two western blot experiments with each primary antibody.

Background: Cofilin and ADF (actin-depolymerization factor) are members of a family of essential conserved small actin-binding proteins that play pivotal roles in cytokinesis, endocytosis, embryonic development, stress response, and tissue regeneration (1). In response to stimuli, cofilin promotes the regeneration of actin filaments by severing preexisting filaments (2). The severing activity of cofilin is inhibited by LIMK or TESK phosphorylation at Ser3 of cofilin (3-5). Phosphorylation at Ser3 also regulates cofilin translocation from the nucleus to the cytoplasm (6).

LIM kinases (LIMK1 and LIMK2) are serine/threonine kinases that have two zinc finger motifs, known as LIM motifs, in their amino-terminal regulatory domains (7). LIM kinases are involved in actin cytoskeletal regulation downstream of Rho-family GTPases, PAKs, and ROCK (8,9). PAK1 and ROCK phosphorylate LIMK1 or LIMK2 at the conserved Thr508 or Thr505 residues in the activation loop, increasing LIMK activity (9-11). Activated LIM kinases inhibit the actin depolymerization activity of cofilin by phosphorylation at the amino-terminal Ser3 residue of cofilin (12,13).

Testis-specific kinase 1 (TESK1) is an LIMK-related protein kinase originally identified to be highly expressed in testes and subsequently shown to be expressed in a wide variety of tissues and cell types (14-17). TESK1 phosphorylates the actin severing protein cofilin at Ser3, inactivating cofilin and thus regulating the organization of the actin cytoskeleton (15). Integrin signaling activates TESK1 activity and leads to stress fiber formation and cell spreading (15,18,19). TESK1 is involved in regulation of ERK signaling through its interaction with Spry2 (20) and regulation of cell spreading through its interaction with the focal adhesion protein actopaxin/α-parvin (18).

Chronophin (CIN, PDXP) is a haloacid dehalogenase phosphatase that dephosphorylates cofilin. Alteration of CIN activity through overexpression of either the wildtype or phosphatase-inactive mutant CIN interferes with actin dynamics, cell morphology and cytokinesis (21).

ROCK (Rho-associated kinase), a family of serine/threonine kinases, is an important downstream target of GTPase Rho and plays an important role in Rho-mediated signaling. Two isoforms of ROCK have been identified (ROCK1 and ROCK2). ROCK is composed of N-terminal catalytic, coiled-coil, and C-terminal PH (pleckstrin homology) domains. The C-terminus of ROCK negatively regulates its kinase activity (22,23). Caspase-3-induced cleavage of ROCK1 and direct cleavage of ROCK2 by granzyme B (grB) activates ROCK and leads to phosphorylation of myosin light chain and inhibition of myosin phosphatase (24). This phosphorylation may account for the mechanism by which Rho regulates cytokinesis, cell motility, cell membrane blebbing during apoptosis, and smooth muscle contraction (25-27).

Slingshot homolog 1 (SSH1) can also dephosphorylate LIMK kinases, suppressing LIMK phosphorylation of cofilin (28). In addition, SSH1 modulates actin dynamics by stabilizing F-actin and promoting actin bundling independent of its cofilin phosphatase activity (29). SSH1 activity is regulated by phosphorylation and protein-protein interaction through various signaling pathways (1). Binding of SSH1 to F-actin stimulates its cofilin phosphatase activity (30).

Specificity/Sensitivity: Cofilin (D3F9) XP® Rabbit mAb detects endogenous levels of total cofilin1 protein. Phospho-Cofilin (Ser3) (77G2) Rabbit mAb detects endogenous levels of cofilin only when phosphorylated at Ser3. LIMK2 (8C11) Rabbit mAb detects endogenous levels of total LIMK2 protein and does not cross-react with LIMK1. TESK1 (D49D4) Rabbit mAb detects endogenous levels of total TESK1 protein. Chronophin/PDXP (C85E3) Rabbit mAb detects endogenous levels of total chronophin/PDXP protein. ROCK1 (C8F7) Rabbit mAb detects endogenous levels of total ROCK1 protein. SSH1 (E1K3W) Rabbit mAb recognizes endogenous levels of total SSH1 protein. Based on the absence of sequence homology, this antibody is not expected to recognize SSH2 or SSH3.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to central residues of human cofilin1 protein, carboxy-terminal residues of human LIMK2 protein, carboxy-terminal residues of human TESK1 protein, the central sequence of human ROCK1 protein; with recombinant mouse MBP-chronophin protein; with a synthetic phosphopeptide corresponding to residues surrounding Ser3 of human cofilin protein; or with residues surrounding Pro1018 of human SSH1 protein.

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.

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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.