Store at -20°C

#8606

Actin Nucleation and Polymerization 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
ARP2 Antibody	3128	20 µl	44 kDa	Rabbit IgG
ARP3 Antibody	4738	20 µl	47 kDa	Rabbit IgG
Profilin-1 (C56B8) Rabbit mAb	3246	20 µl	15 kDa	Rabbit IgG
Phospho-Rac1/cdc42 (Ser71) Antibody	2461	20 µl	28 kDa	Rabbit IgG
Rac1/Cdc42 Antibody	4651	20 µl	21 kDa	Rabbit IgG
N-WASP (30D10) Rabbit mAb	4848	20 µl	65 kDa	Rabbit IgG
WAVE-2 (D2C8) XP® Rabbit mAb	3659	20 µl	80 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.

Description: The Actin Nucleation and Polymerization Antibody Sampler Kit provides an economical means to evaluate the presence and status of actin nucleation and polymerization. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Actin nucleation is the process of forming new actin filaments and is necessary to stimulate actin polymerization. Actin polymerization is vital for cell motility, cell division, and cell adhesion. Rac and Cdc42, members of the Rho-GTPase family, play key roles in actin dynamics, membrane trafficking, transcriptional regulation, cell growth, and development (1). GTP binding stimulates the activity of Rac/Cdc42, and the hydrolysis of GTP to GDP through the intrinsic GTPase activity of Rac/Cdc42, rendering it inactive. GTP hydrolysis is aided by GTPase activating proteins (GAPs), while exchange of GDP for GTP is facilitated by guanine nucleotide exchange factors (GEFs). Another level of regulation is achieved through binding of RhoGDI, a guanine dissociation inhibitor, which retains Rho family GTPases, including Rac and Cdc42, in their inactive GDP-bound state (2,3). Hematopoietic WASP and ubiquitously expressed N-WASP are autoinhibited in unstimulated cells. Upon stimulation they are activated by Cdc42, which relieves the autoinhibition in conjunction with phosphatidyl inositol 4,5-bisphosphate (4). Three WAVE (Wasf, SCAR) family proteins are similar in sequence to WASP and N-WASP, but lack the WASP/N-WASP autoinhibition domains and are indirectly activated by Rac (4). WAVE-2 is widely distributed, while WAVE-1 and WAVE-3 are strongly expressed in the brain (5). The highly conserved ARP2/3 complex is an important actin nucleation protein complex consisting of ARP2, ARP3, and ARPC1-5. The ARP2/3 complex promotes branching of existing actin filaments and formation of daughter filaments following activation by nucleation-promoting factors, such as WASP/WAVE or cortactin (6). Profilins are conserved actin binding proteins that affect the rate of actin polymerization by binding actin monomers and promoting exchange of ADP for ATP (reviewed in 7). Profilins bind to proteins involved in the regulation of actin dynamics including paladin (8), dynamin-I (9), VASP (10), and N-WASP (11).

Specificity/Sensitivity: ARP2 Antibody recognizes endogenous levels of total ARP2 protein. ARP3 Antibody recognizes endogenous levels of total ARP3 protein. This antibody does not cross-react with endogenous ARP2. Profilin-1 (C56B8) Rabbit mAb recognizes endogenous levels of total profilin-1 protein. This antibody may cross-react with profilin-2. Phospho-Rac1/cdc42 (Ser71) Antibody recognizes endogenous levels of Rac1/cdc42 only when phosphorylated at serine 71. This antibody may also detect phospho-RhoA (Ser73). Rac1/Cdc42 Antibody recognizes endogenous levels of total rac1 and cdc42 proteins. Based on sequence similarities, this antibody may also detect rac2 and rac3; this antibody does not cross-react with RhoA, RhoB, or RhoC. N-WASP (30D10) Rabbit mAb recognizes endogenous levels of total N-WASP protein. This antibody does not cross-react wth the hematopietic protein, WASP. WAVE-2 (D2C8) XP® Rabbit mAb recognizes endogenous levels of total WAVE-2 protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human profilin-1 protein, the sequence of human N-WASP protein, or central residues of human WAVE-2 protein.

Activation state specific polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser71 of human Rac1/cdc42 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the amino termini of human rac1 and cdc42 proteins, the sequence of human ARP2 protein, or the amino terminus of human ARP3 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Wennerberg, K. and Der, C.J. (2004) *J Cell Sci* 117, 1301-12.
(2) Bernards, A. and Settleman, J. (2004) *Trends Cell Biol* 14, 377-85.
(3) Rossman, K.L. et al. (2005) *Nat Rev Mol Cell Biol* 6, 167-80.
(4) Millard, T.H. et al. (2004) *Biochem J* 380, 1-17.
(5) Suetsugu, S. et al. (1999) *Biochem Biophys Res Commun* 260, 296-302.
(6) Goley, E.D. and Welch, M.D. (2006) *Nat Rev Mol Cell Biol* 7, 713-26.
(7) Witke, W. (2004) *Trends Cell Biol* 14, 461-9.
(8) Boukhelifa, M. et al. (2006) *FEBS J* 273, 26-33.
(9) Gareus, R. et al. (2006) *J Biol Chem* 281, 2803-11.
(10) Reinhard, M. et al. (1995) *EMBO J* 14, 1583-9.
(11) Suetsugu, S. et al. (1998) *EMBO J* 17, 6516-26.

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.

#8606

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.