

#3114 Store at -20°C

Phospho-VASP (Ser239) Antibody

✓ 100 µl
(10 Western mini-blots)



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Support ■ 877-678-TECH (8324)
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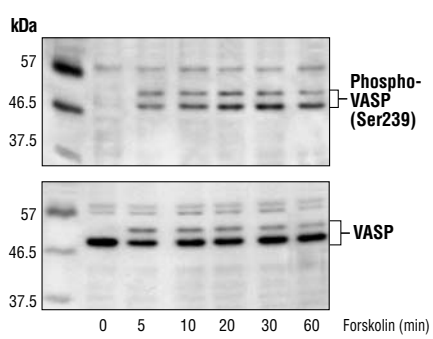
rev. 10/16/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, M, R, Mk	48, 51 kDa	Rabbit

Background: Vasodilator-stimulated phosphoprotein (VASP) was originally characterized as a substrate of both cGMP- and cAMP-dependent kinases (PKG and PKA, or cGPK and cAPK, respectively) (1). It is now believed that VASP belongs to the Ena/VASP family of adaptor proteins linking the cytoskeletal system to the signal transduction pathways and that it functions in cytoskeletal organization, fibroblast migration, platelet activation and axon guidance (2,3). Three phosphorylation sites, Ser157, Ser239 and Thr278, have been identified. Ser239 is the major PKG phosphorylation site while Ser157 is the major PKA phosphorylation site (4). Evidence suggests that VASP phosphorylation reduces its association with actin and has a negative effect on actin polymerization (5). Phosphorylation at Ser239 of VASP is a useful marker for monitoring PKG activation and signaling (6,7).

Specificity/Sensitivity: Phospho-VASP (Ser239) Antibody detects endogenous levels of VASP only when phosphorylated at Ser239. The antibody does not cross-react with phosphorylated VASP homologues, such as Mena.

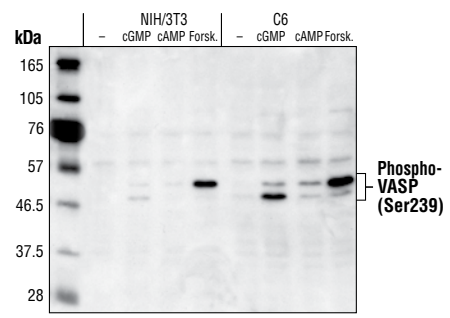


Western blot analysis of extracts from A431 cells stimulated with forskolin for indicated times using Phospho-VASP (Ser239) Antibody (upper) and VASP Antibody #3112 (lower).

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser239 of human VASP. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- (1) Butt, E. et al. (1994) *J. Biol. Chem.* 269, 14509–14517.
- (2) Ball, L.J. et al. (2000) *EMBO J.* 19, 4903–4914.
- (3) Machesky, L.M. et al. (2000) *Cell* 101, 685–688.
- (4) Smolenski, A. et al. (1998) *J. Biol. Chem.* 273, 20029–20035.
- (5) Harbeck, B. et al. (2000) *J. Biol. Chem.* 275, 30817–30825.
- (6) Oelze, M. et al. (2000) *Circ. Res.* 87, 999–1005.
- (7) Lawrence, D.W. et al. (2001) *J. Immunol.* 166, 5550–5556.



Western blot analysis of extracts from NIH/3T3 and C6 cells, untreated, 8-Br-cGMP-treated, 8-Br-cAMP-treated or forskolin-treated as indicated using Phospho-VASP (Ser239) Antibody.

IMPORTANT: 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.

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 antibody.

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:
Western blotting 1:1000

Companion Products:
Phospho-VASP (Ser157) Antibody #3111
VASP Antibody #3112
VASP (9A2) Rabbit mAb #3132
VASP (A290) Antibody #3120
Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
Anti-rabbit IgG, HRP-linked Antibody #7074
Prestained Protein Marker, Broad Range (Premixed Format) #7720
Biotinylated Protein Ladder #7727
20X LumiGLO® Reagent and 20X Peroxide #7003

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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- 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.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- 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 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

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.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- 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.

- 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.