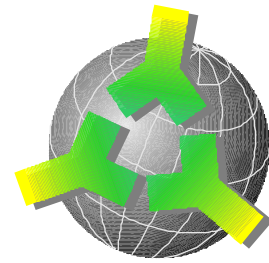


anti-VASP

mouse monoclonal antibody IE273

Lot: 633

catalog #: 0016-05



immunoGlobe
Antikörpertechnik GmbH

Background information

VASP (vasodilator stimulated phosphoprotein) is a proline-rich²¹ protein substrate of cAMP- and cGMP-dependent protein kinases^{18,20,26-43}. Phosphorylation of VASP at Ser-157 causes a mobility shift in SDS gel electrophoresis from 46 to 50 kDa²⁵, which has been used as a convenient marker to monitor cyclic nucleotide-dependent protein kinase activity^{16,18,20,26-40}. VASP is the founding member of the Ena-VASP protein family, comprising the Drosophila protein Enabled (Ena)¹⁹, its mouse homologue Mena (mammalian Enabled)⁹, and mouse EVL (Ena-VASP-like protein)⁹. With these proteins VASP shares a conserved overall domain organization^{9,21}: a) the conserved N-terminal Ena-VASP homology domain 1 (EVH1), which mediates binding to a novel proline-rich motif^{1,6,10}, b) a more divergent proline-rich central domain (which is responsible for profilin binding)^{4,5,24}, and c) a conserved C-terminal EVH2 domain. Particularly high VASP levels are present in platelets^{33,36,41}, although VASP is expressed in a variety of mammalian cell types and tissues^{11,32,33,36,41}. In cultured cells, VASP is associated with focal adhesions, cell-cell contacts, microfilaments, and highly dynamic membrane regions^{2,24,36}. From *in vitro* binding data VASP has been suggested to link profilin to zyxin²⁵, vinculin^{6,10}, and the *Listeria spp.* surface protein ActA^{8,17,22}, respectively. Functional evidence indicates that VASP is a crucial factor involved in the enhancement of actin filament formation and the actin-dependent motility of intracellular bacterial pathogens^{1-3,10,12-14,23}.

Antibody preparation and storage

Clone⁴⁴: IE273, isotype IgG₁. 50 µg of purified antibody in PBS, with 1 mg/ml BSA and 0.02% (w/v) NaN₃. Antibody concentration: 500 µg/ml. Vials have been overfilled by 10% to ensure complete recovery of the specified amount. Short term storage at 4°C, stable for one year from date of shipment when stored at -20°C. Avoid repeated freezing and thawing! Do not store in "frost-free" freezers.

Antigen

VASP purified⁴² from human platelets.

Species cross-reactivity

human, porcine, and bovine VASP

Specificity

The antibody recognizes both the 46 kDa (Ser-157 dephospho) and 50 kDa (Ser-157 phospho) form of VASP.

Applications

Western (immuno) blotting (0.1-0.25 µg/ml; dilution 1:2000 - 1:5000), immunofluorescence of formaldehyde fixed cells (1-5 µg/ml; dilution 1:100 - 1:500), immunoprecipitation with anti-mouse secondary antibody.

All dilutions refer to the analysis of cells and tissues with intermediate to high levels of VASP expression and must be viewed as approximate. The antibody should be titrated for each individual application.

Positive control

Human platelet protein (500 µg), supplied at 5 mg/ml in SDS-stop solution (100 mM NaCl, 73 mM Tris/HCl pH 6.7, 10 mM DTT, 8 mM EDTA, 5 % [v/v] glycerol, 2% [w/v] SDS, 10 µg/ml Bromophenol Blue sodium salt). Use 5 µl (25 µg) per lane.

Related products

- mouse monoclonal antibody IE273 to VASP, carrier-free, 1 mg (catalog # 0016-blk)
- affinity purified rabbit antibody IG731 to human VASP, 25 µg (catalog # 0012-02)
- positive control: human platelet protein in SDS-sample buffer, 500 µg (catalog # 8010-50)

References

(*: papers referencing the VASP mAb IE273)

- * [1] Niebuhr et al. (1997) A novel proline-rich motif present in ActA of *Listeria monocytogenes* and cytoskeletal proteins is the ligand for the EVH1 domain, a protein module present in the Ena/VASP family. *EMBO J.*, **16**:5433-5444.
- [2] Reinhard et al. (1997) VASP. *Guidebook to the cytoskeleton and motor proteins*. In press.

./.2

- [3] Higley & Way (1997) Actin and cell pathogenesis. *Curr. Opin. Cell Biol.* **9**:62-69.
- [4] Kang et al. (1997) Profilin interacts with the Gly-Pro-Pro-Pro-Pro-Pro sequences of vasodilator-stimulated phosphoprotein (VASP): Implications for actin-based *Listeria* motility. *Biochemistry* **36**:8384-8392.
- * [5] Lambrechts et al. (1997) The mammalian profilin isoforms display complimentary affinities for PIP₂ and proline-rich sequences. *EMBO J.* **16**:484-494.
- [6] Brindle et al. (1996) The focal adhesion vasodilator-stimulated phosphoprotein (VASP) binds to the proline-rich domain in vinculin. *Biochem. J.* **318**:753-757.
- [7] Dutarre et al. (1997) Cytokinesis arrest and redistribution of actin cytoskeleton regulatory components in cells expressing the Rho GTPase CDC42HS. *J. Cell Sci.* **109**:367-377.
- [8] Gerstel et al. (1996) The ActA polypeptides of *Listeria ivanovii* and *Listeria monocytogenes* harbor related binding sites for host microfilament proteins. *Infect. Immunif.* **64**:1929-1936.
- [9] Gertler et al. (1996) Mena, a relative of VASP and *Drosophila Enabled*, is implicated in the control of microfilament dynamics. *Cell* **87**:227-239.
- [10] Reinhard et al. (1996) VASP interaction with vinculin: a recurring theme of interactions with proline-rich motifs. *FEBS Lett.* **399**:103-107.
- [11] Markert et al. (1996) High expression of the focal adhesion and microfilament associated protein VASP in vascular smooth muscle and endothelial cells of the intact human vessel wall. *Basic Res. Cardiol.* **91**:337-343.
- [12] Smith et al. (1996) The tandem repeat domain in the *Listeria monocytogenes* ActA protein controls the rate of actin based motility, the percentage of moving bacteria, and the localization of vasodilator stimulated phosphoprotein and profilin. *J. Cell Biol.* **135**:647-660.
- [13] Southwick & Purich (1996) Intracellular pathogenesis of listeriosis. *New Engl. J. Med.* **334**:770-776.
- [14] Zeile et al. (1996) Recognition of two classes of oligoproline sequences in profilin-mediated acceleration of actin-based *Shigella* motility. *J. Cell. Biol.* **133**:49-59.
- [15] Zimmer et al. (1996) Cloning of the VASP (vasodilator-stimulated phosphoprotein) genes in human and mouse: structure, sequence, and chromosomal localization. *Genomics* **36**:227-233.
- [16] Abel et al. (1995) Dephosphorylation of the focal adhesion protein VASP in vitro and in intact human platelets. *FEBS Lett.* **370**:184-188.
- [17] Chakraborty et al. (1995) A focal adhesion factor directly linking intracellularly motile *Listeria monocytogenes* and *Listeria ivanovii* to the actin-based cytoskeleton of mammalian cells. *EMBO J.* **14**:1314-1321.
- [18] Draijer et al. (1995) Expression of cGMP-dependent protein kinase I and phosphorylation of its substrate, vasodilator-stimulated phosphoprotein, in human endothelial cells of different origin. *Circ. Res.* **77**:897-905.
- [19] Gertler et al. (1995) *Enabled*, a dosage-sensitive suppressor of mutations in the *Drosophila* Abl tyrosine kinase, encodes an Abl substrate with SH3-domain binding properties. *Genes Dev* **9**, 521-33.
- [20] Grunberg et al. (1995) Platelet rap1B phosphorylation is a sensitive marker for the action of cyclic AMP- and cyclic GMP-increasing platelet inhibitors and vasodilators. *J. Cardiovasc. Pharmacol.* **25**:545-551.
- [21] Haffner et al. (1995) Molecular cloning, structural analysis, and functional expression of the proline-rich focal adhesion and microfilament-associated protein VASP. *EMBO J.* **14**:19-27.
- [22] Pistor et al. (1995) The bacterial actin nucleator protein ActA of *Listeria monocytogenes* contains multiple binding sites for host microfilament proteins. *Curr. Biol.* **5**:517-525.
- [23] Pollard (1995) Actin cytoskeleton. Missing link for intracellular bacterial motility? *Curr. Biol.* **5**:837-840.
- [24] Reinhard et al. (1995) The proline-rich focal adhesion and microfilament protein VASP is a ligand for profilins. *EMBO J.* **14**:1583-1589.
- [25] Reinhard et al. (1995) Identification, purification, and characterization of a zyxin-related protein which binds the focal adhesion and microfilament protein VASP. *Proc. Natl. Acad. Sci. USA* **92**:7956-7960.
- [26] Butt et al. (1994) cAMP- and cGMP-dependent protein kinase phosphorylation sites of the focal adhesion vasodilator-stimulated phosphoprotein (VASP) in vitro and in intact human platelets. *J. Biol. Chem.* **269**:14509-14517.
- [27] Horstrup et al. (1994) Phosphorylation of focal adhesion vasodilator-stimulated phosphoprotein at Ser157 in intact human platelets correlates with fibrinogen receptor inhibition. *Eur. J. Biochem.* **225**:21-27.
- [28] Kruse et al. (1994) Formation of biologically active autacoids is regulated by calcium influx in endothelial cells. *Arterioscler. Thromb.* **14**:1821-1828.
- [29] Nolte et al. (1994) Synergistic phosphorylation of the focal adhesion-associated Vasodilator-stimulated phosphoprotein in intact human platelets in response to cGMP- and cAMP-elevating platelet inhibitors. *Biochem. Pharmacol.* **48**:1569-1575.
- [30] Pohl et al. (1994) Endothelium-dependent phosphorylation of vasodilator-stimulated protein in platelets during coronary passage. *Am. J. Physiol.* **266**:H606-612.
- [31] Eigenthaler et al. (1993) Defective nitrovasodilator-stimulated protein phosphorylation and calcium regulation in cGMP-dependent protein kinase-deficient human platelets of chronic myelocytic leukemia. *J. Biol. Chem.* **268**:13526-31.
- [32] Walter et al. (1993) Role of cyclic nucleotide-dependent protein kinases and their common substrate VASP in the regulation of human platelets. *Adv. Exp. Med. Biol.* **344**:237-249.
- [33] Eigenthaler et al. (1992) Concentration and regulation of cyclic nucleotides, cyclic-nucleotide-dependent protein kinases and one of their major substrates in human platelets. Estimating the rate of cAMP-regulated and cGMP-regulated protein phosphorylation in intact cells. *Eur. J. Biochem.* **205**:471-481.
- [34] Geiger et al. (1992) Role of cGMP and cGMP-dependent protein kinases in nitrovasodilator inhibition of agonist-evoked calcium elevation in human platelets. *Proc. Natl. Acad. Sci. USA* **89**:1031-1035.
- [35] Halbrügge et al. (1992) Protein phosphorylation regulated by cyclic nucleotide-dependent protein kinases in cell extracts and in intact human lymphocytes. *Cell. Signalling* **4**:189-199.
- [36] Reinhard et al. (1992) The 46/50 kDa phosphoprotein VASP purified from human platelets is a novel protein associated with actin filaments and focal contacts. *EMBO J.* **11**:2063-2070.
- [37] Nolte et al. (1991) Comparison of vasodilatory prostaglandins with respect to cAMP-mediated phosphorylation of a target substrate in intact human platelets. *Biochem. Pharmacol.* **42**:253-262.
- [38] Nolte et al. (1991) Endothelial cell-dependent phosphorylation of a platelet protein mediated by cAMP- and cGMP-elevating factors. *J. Biol. Chem.* **266**:14808-14812.
- [39] Sandberg et al. (1991) Characterization of Sp-5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole-3',5'-monophosphorothioate (Sp-5,6-DCI-cBiMPS) as a potent and specific activator of cyclic-AMP-dependent protein kinase in cell extracts and intact cells. *Biochem. J.* **279**:521-527.
- [40] Halbrügge et al. (1990) Stoichiometric and reversible phosphorylation of a 46 kDa protein in human platelets in response to cGMP- and cAMP-elevating vasodilators. *J. Biol. Chem.* **265**:3088-3093.
- [41] Halbrügge & Walter (1990) Analysis, purification and properties of a 50,000-dalton membrane-associated phosphoprotein from human platelets. *J. Chromatogr.* **521**:335-343.
- [42] Halbrügge & Walter (1989) Purification of a vasodilator-regulated phosphoprotein from human platelets. *Eur. J. Biochem.* **185**:41-50.
- [43] Waldmann et al. (1987) Vasodilator-stimulated protein phosphorylation in platelets by cAMP- and cGMP-dependent protein kinases. *Eur. J. Biochem.* **167**:441-448.*
- [44] Abel et al. (1996) Monoclonal antibodies against the focal adhesion protein VASP revealing epitopes involved in the interaction with two VASP binding proteins and VASP phosphorylation. *Eur. J. Cell Biol.* **69** (Suppl. 42):39a.

for research use only — not for human, in vivo, diagnostic, therapeutic or other uses