

# anti-VASP

## affinity purified rabbit antibody IG731

Lot: # 881

data sheet 0505— catalog # 0012-02



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### Background information

VASP (vasodilator stimulated phosphoprotein) is a proline-rich<sup>28</sup> protein substrate of cAMP- and cGMP-dependent protein kinases<sup>27,31-45</sup>. Phosphorylation of VASP at Ser-157 causes a mobility shift in SDS gel electrophoresis from 46 to 50 kDa<sup>31,44</sup>, which has been used as a convenient marker to monitor cyclic nucleotide-dependent protein kinase activity<sup>25,27,31-44</sup>. VASP is the founding member of the Ena-VASP protein family, comprising the *Drosophila* protein Enabled (Ena), its mouse homologue Mena (mammalian Enabled), and mouse EVL (Ena-VASP-like protein)<sup>16</sup>. With these proteins VASP shares a conserved overall domain organization: a) the conserved N-terminal Ena-VASP homology domain 1 (EVH1), which mediates binding to a defined proline-rich motif<sup>19</sup>, b) a more divergent proline-rich central domain (which is responsible for profilin, SH3, and WW domain binding)<sup>16</sup>, and c) a conserved C-terminal EVH2 oligomerization<sup>18</sup> domain. VASP is expressed in a variety of mammalian cell types and tissues<sup>23,36,37,40,45</sup>. In cultured cells, VASP is associated with focal adhesions, cell-cell contacts, microfilaments, and highly dynamic membrane regions<sup>16,17,30,40</sup>. Functional evidence indicates that VASP is a crucial factor involved in the regulation of actin filament dynamics and actin-dependent motility of cells and intracellular bacterial pathogens<sup>12</sup> (for a review see Refs.<sup>1,5,6,8,11,13,14,16,17,46</sup>).

### Antibody preparation and storage

25 µg (100 µl) of purified antibody in PBS, with 1 mg/ml BSA, with 0.02% NaN<sub>3</sub>. Antibody concentration: 250 µg/ml. Vials have been overfilled by 10% to ensure complete recovery of the specified amount. Stable for one year from date of shipment when stored at -20°C.

### Antigen

The antibody was raised against human His<sub>6</sub>-VASP and has been affinity purified on the antigen.

### Species cross-reactivity

Human, mouse, porcine

### 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 µg/ml; dilution 1:2.500), immunofluorescence of formaldehyde fixed cells (1 µg/ml; dilution 1:250), immunoprecipitation (2 µg/ml; dilution 1:125). All dilutions refer to the analysis of cells and tissues with intermediate to high levels of human 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 sample buffer. Use 5 µl (25 µg) per lane.

### Related products

- monoclonal antibody IE273 to human VASP, 50 µg (catalog # 0016-05)
- positive control: human platelet protein in SDS sample buffer, 500 µg (catalog # 8010-50)

### References

- [1] Gomez & Robles (2004) The great escape; phosphorylation of Ena/VASP by PKA promotes filopodial formation. *Neuron* **42**:1-3.
- [2] Lin et al. (2004) Effect of cell passage and density on protein kinase G expression and activation in vascular smooth muscle cells. *J. Cell. Biochem.* **92**:104-12.
- [3] Spinardi et al. (2004) A dynamic podosome-like structure of epithelial cells. *Exp. Cell Res.* **295**:360-74.  
Zhuang et al. (2004) Vasodilator-stimulated phosphoprotein activation of serum-response element-dependent transcription occurs downstream of Rho A and is inhibited by cGMP-dependent protein kinase phosphorylation. *J. Biol. Chem.* **279**:10397-407.
- [4] Gustavsson et al. (2004) Temporal dissection of beta 1-integrin signaling indicates a role for p130Cas/Crk in filopodia formation. *J. Biol. Chem.* **279**:22893-901
- [5] Krause et al. (2003) Ena/VASP proteins: regulators of the actin cytoskeleton and cell migration. *Annu. Rev. Cell. Dev. Biol.* **19**:541-64.
- [6] Kwiatkowski et al. (2003) Function and regulation of Ena/VASP proteins. *Trends Cell Biol.* **13**:386-92.
- [7] Grosse et al. (2003) A role for VASP in RhoA-Diaphanous signalling to actin dynamics and SRF activity. *EMBO J.* **22**:3050-61.
- [8] Kwiatkowski et al. (2003) Function and regulation of Ena/VASP proteins. *Trends Cell Biol.* **13**:386-92.

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- [9] Anderson et al. (2003) Linked regulation of motility and integrin function in activated migrating neutrophils revealed by interference in remodelling of the cytoskeleton. *Cell Motil. Cytoskeleton* **54**:135-46.
- [10] Sudo et al. (2003) Phosphorylation of the vasodilator-stimulated phosphoprotein (VASP) by the anti-platelet drug, cilostazol, in platelets. *Platelets*. **14**:381-90.
- [11] Samarín et al. (2003) How VASP enhances actin-based motility. *J. Cell Biol.* **163**:131-42.
- [12] Auerbuch et al. (2003) Ena/VASP proteins contribute to *Listeria monocytogenes* pathogenesis by controlling temporal and spatial persistence of bacterial actin-based motility. *Mol. Microbiol.* **49**:1361-75.
- [13] Krause et al. (2002) The Ena/VASP enigma. *J. Cell Sci.* **115**:4721-6.
- [14] Renfranz & Beckerle (2002) Doing (F/L)PPPPs: EVH1 domains and their proline-rich partners in cell polarity and migration. *Curr. Opin. Cell Biol.* **14**:88-103.
- [15] Garcia-Arguinzonis et al. (2002) Increased spreading, Rac/p21-activated kinase (PAK) activity, and compromised cell motility in cells deficient in vasodilator-stimulated phosphoprotein (VASP). *J. Biol. Chem.* **277**:45604-10.
- [16] Reinhard et al. (2001) Actin-based motility: stop and go with Ena/VASP proteins. *Trends Biochem. Sci.* **26**:243-9.
- [17] Vasioukhin & Fuchs (2001) Actin dynamics and cell-cell adhesion in epithelia. *Curr. Opin. Cell Biol.* **13**:76-84.
- [18] Bachmann et al. (1999) The EVH2 domain of the vasodilator-stimulated phosphoprotein mediates tetramerization, F-actin binding, and actin bundle formation. *J. Biol. Chem.* **274**:23549-57.
- [19] 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.
- [20] Lambrechts et al. (1997) The mammalian profilin isoforms display complementary affinities for PIP<sub>2</sub> and proline-rich sequences. *EMBO J.* **16**:484-494.
- [21] Dutartre et al. (1996) Cytokinesis arrest and redistribution of actin cytoskeleton regulatory components in cells expressing the Rho GTPase CDC42HS. *J. Cell Sci.* **109**:367-377.
- [22] Gerstel et al. (1996) The ActA polypeptides of *Listeria ivanovii* and *Listeria monocytogenes* harbor related binding sites for host microfilament proteins. *Infect. Immunit.* **64**:1929-1936.
- [23] 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.
- [24] 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.
- [25] Abel et al. (1995) Dephosphorylation of the focal adhesion protein VASP in vitro and in intact human platelets. *FEBS Lett.* **370**:184-188.
- [26] 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.
- [27] 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.
- [28] 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.
- [29] 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.
- [30] Reinhard et al. (1995) The proline-rich focal adhesion and microfilament protein VASP is a ligand for profilins. *EMBO J.* **14**:1583-1589.
- [31] 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.
- [32] 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.
- [33] 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.
- [34] Pohl et al. (1994) Endothelium-dependent phosphorylation of vasodilator-stimulated protein in platelets during coronary passage. *Am. J. Physiol.* **266**:H606-612.
- [35] 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.
- [36] 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.
- [37] 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.
- [38] 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.
- [39] 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.
- [40] 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.
- [41] 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.
- [42] 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.
- [43] 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.
- [44] 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.
- [45] Halbrügge & Walter (1990) Analysis, purification and properties of a 50,000-dalton membrane-associated phosphoprotein from human platelets. *J. Chromatogr.* **521**:335-343.
- [46] Legg & Machesky (2004) MRL proteins: leading Ena/VASP to Ras GTPases. *Nature Cell Biol.* **6**:1015-1017.

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