# anti-VASP mouse monoclonal antibody IE273

Lot: 991

catalog #: 0016-05



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# **Background information**

VASP (vasodilator stimulated phosphoprotein) is a proline-rich<sup>21</sup> protein substrate of cAMP- and kinases<sup>18,20,26-43</sup> cGMP-dependent protein Phosphorylation of VASP at Ser-157 causes a mobility shift in SDS gel electrophoresis from 46 to 50 kDa<sup>25</sup>, which has been used as a convenient marker to monitor cyclic nucleotidedependent protein kinase activity<sup>16,18,20,26-40</sup>. VASP is the founding member of the Ena-VASP protein family, comprising the Drosophila protein Enabled (Ena)<sup>19</sup>, its mouse homologue Mena (mammalian Enabled)<sup>9</sup>, and mouse EVL (Ena-VASP-like protein)<sup>9</sup>. With these proteins VASP shares conserved overall domain а organization<sup>9,21</sup>: a) the conserved N-terminal Ena-VASP homology domain 1 (EVH1), which mediates binding to a novel proline-rich motif<sup>1,6,10</sup>. 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<sup>33,36,41</sup>, although VASP is expressed in a variety of mammalian cell types and tissues<sup>11,32,33,36,41</sup>. In cultured cells, VASP is associated with focal adhesions, cell-cell contacts, microfilaments, and highly dynamic membrane regions<sup>2,24,36</sup>. From *in vitro* binding data VASP has been suggested to link profilin to zyxin<sup>25</sup>, vinculin<sup>6,10</sup>, and the *Listeria spp.* surface protein ActA<sup>8,17,22</sup>, 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<sup>1-3,10,12-14,23</sup>.

# Antibody preparation and storage

Clone<sup>44</sup>: IE273, isotype  $IgG_1$ . 50 µg of purified antibody in PBS with 2 mg/ml BSA and 0.02% (w/v) NaN<sub>3</sub>. 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 <u>not</u> store in "frost-free" freezers.

# Antigen

VASP purified<sup>42</sup> 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 (0.01-0.25  $\mu$ g/ml; dilution 1:2,000 - 1:50,000), immunofluorescence of formaldehyde fixed cells (1-5  $\mu$ g/ml; dilution 1:100 - 1:500), immunoprecipitation with antimouse 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  $\mu$ g), 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  $\mu$ g/ml Bromophenol Blue sodium salt). Use 5  $\mu$ l (25  $\mu$ g) per lane.

### **Related products**

- rabbit antiserum to human VASP, 100 µl (catalog # 0017-10)
- affinity purified rabbit antibody to human VASP, 25 μg (catalog # 0012-02)
- positive control: human platelet protein in SDSsample buffer, 500 µg (catalog # 8010-50)

## References

### (\*: papers referencing the VASP mAb IE273)

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