

#### DESCRIPTION

|                           |  |
|---------------------------|--|
| <b>Species Reactivity</b> | Human  |
| <b>Specificity</b>        | Detects human ROBO4 in direct ELISAs and Western blots. In direct ELISAs, no cross-reactivity with recombinant rat ROBO1, recombinant human (rh) ROBO2, or rhROBO3 is observed.  |
| <b>Source</b>             | Monoclonal Mouse IgG <sub>2B</sub> Clone # 265721  |
| <b>Purification</b>       | Protein A or G purified from hybridoma culture supernatant   |
| <b>Immunogen</b>          | Mouse myeloma cell line NS0-derived recombinant human ROBO4<br>Gln28-Arg467<br>Accession # Q8WZ75  |
| <b>Conjugate</b>          | Alexa Fluor 488<br>Excitation Wavelength: 488 nm<br>Emission Wavelength: 515-545 nm  |
| <b>Formulation</b>        | Supplied 0.2 mg/mL in a saline solution containing BSA and Sodium Azide. See Certificate of Analysis for details.<br><br>*Contains <0.1% Sodium Azide, which is not hazardous at this concentration according to GHS classifications. Refer to the Safety Data Sheet (SDS) for additional information and handling instructions. |

#### APPLICATIONS

**Please Note:** Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

|                       | <b>Recommended Concentration</b> | <b>Sample</b>                                |
|-----------------------|----------------------------------|--|
| <b>Flow Cytometry</b> | 0.25-1 µg/10 <sup>6</sup> cells  | HUVEC human umbilical vein endothelial cells |

#### PREPARATION AND STORAGE

|                                |  |
|--------------------------------|--|
| <b>Shipping</b>                | The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.                                    |
| <b>Stability &amp; Storage</b> | <b>Protect from light. Do not freeze.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, 2 to 8 °C as supplied.</li> </ul> |

#### BACKGROUND

ROBO4, also called magic roundabout, is a ~150 kDa glycoprotein belonging to the ROBO family (1). ROBOs are molecular guidance receptors that typically interact with Slit ligands to regulate axon guidance and neuronal migration (2). Unlike other family members, ROBO4 is mainly restricted to the vascular endothelium (1, 2). Expression in early hematopoietic progenitors is also reported (3). The human ROBO4 cDNA encodes 1012 amino acids (aa), including a 27 aa signal sequence, a 440 aa extracellular domain (ECD) containing two C2-type Ig domains and two fibronectin type III (FNIII) domains, a transmembrane domain and an intracellular domain. ROBO4 diverges from other ROBO proteins in the number of Ig, FNIII and cytoplasmic CC domains (1, 4). Within the ECD, human ROBO4 shares 80%, 80%, 87% and 88% aa identity with mouse, rat, bovine and canine ROBO4, respectively. Vascular endothelial ROBO4 is expressed at highest levels in during development and vascular remodeling, including tumor angiogenesis (1, 2, 4-6). It is proposed to contribute to vascular stability. Consistent with this, endogenous ROBO4 is concentrated in the vascular stalk and sprouts rather than tip cells and appears to protect newly formed blood vessels against VEGF-induced vascular leak (6-9). ROBO4 binding of Slit proteins has been variably reported, and when detected may be mediated by ROBO4/ROBO1 heterodimers (2, 4-7, 9-13). ROBO4 is also variably reported to stimulate or inhibit cell migration or filopodia formation (2, 4-13). Effects on cell movement may be mediated through intracellular binding of WASP-, Ras/Rac/Rho-, Mena-, Src- or Paxillin-related proteins, all of which affect the cytoskeleton (5-7, 10-12). Recombinant soluble ROBO4 ECD can antagonize endothelial cell migration and in-vivo angiogenesis (13).

#### References:

1. Huminiecki, L. *et al.* (2002) *Genomics* **79**:547.
2. Legg, J.A. *et al.* (2008) *Angiogenesis* **11**:13.
3. Shibata, F. *et al.* (2009) *Stem Cells* **27**:183.
4. Park, K.W. *et al.* (2003) *Dev. Biol.* **261**:251.
5. Seth, P. *et al.* (2005) *Biochem. Biophys. Res. Commun.* **332**:533.
6. Jones, C.A. *et al.* (2008) *Nat. Med.* **14**:448.
7. Jones, C.A. *et al.* (2009) *Nat. Cell Biol.* **11**:1325.
8. Chen, H. *et al.* (2010) *Adv. Exp. Med. Biol.* **664**:457.
9. London, N.R. *et al.* (2009) *J. Thromb. Haemost.* **7**:57.
10. Verissimo, A.R. *et al.* (2009) *Biochem. Soc. Trans.* **37**:1214.
11. Sheldon, H. *et al.* (2009) *FASEB J.* **23**:513.
12. Kaur, S. *et al.* (2008) *BMC Cell Biol.* **9**:61.
13. Suchting, S. *et al.* (2005) *FASEB J.* **19**:121.

**PRODUCT SPECIFIC NOTICES**

This product is provided under an agreement between Life Technologies Corporation and R&D Systems, Inc, and the manufacture, use, sale or import of this product is subject to one or more US patents and corresponding non-US equivalents, owned by Life Technologies Corporation and its affiliates. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components (1) in manufacturing; (2) to provide a service, information, or data to an unaffiliated third party for payment; (3) for therapeutic, diagnostic or prophylactic purposes; (4) to resell, sell, or otherwise transfer this product or its components to any third party, or for any other commercial purpose. Life Technologies Corporation will not assert a claim against the buyer of the infringement of the above patents based on the manufacture, use or sale of a commercial product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, Cell Analysis Business Unit, Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.