

**DESCRIPTION**

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human EphB4 in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant mouse (m) EphB4 is observed and less than 1% cross-reactivity with recombinant human (rh) EphA4, rhEphA3, rhEphB2, and rhEphB3 is observed.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant human EphB4 Leu16-Ala539 Accession # P54760
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

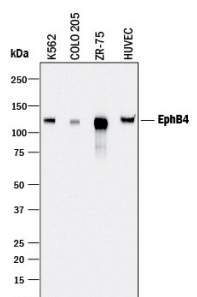
**APPLICATIONS**

**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>Western Blot</b>	2 µg/mL	See Below
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells	See Below
<b>Immunohistochemistry</b>	5-15 µg/mL	See Below
<b>Simple Western</b>	50 µg/mL	See Below
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

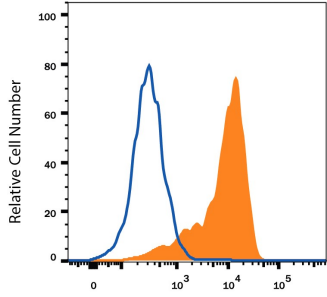
**DATA**

**Western Blot**



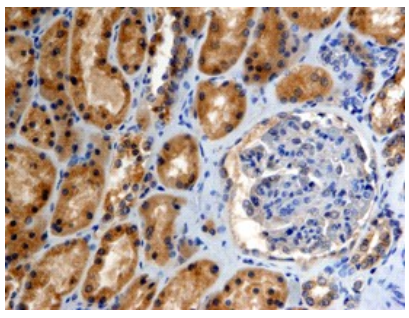
**Detection of Human EphB4 by Western Blot.** Western blot shows lysates of K562 human chronic myelogenous leukemia cell line, COLO 205 human colorectal adenocarcinoma cell line, ZR-75 human breast cancer cell line, and HUVEC human umbilical vein endothelial cells. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human EphB4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3038) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for EphB4 at approximately 120 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Flow Cytometry**



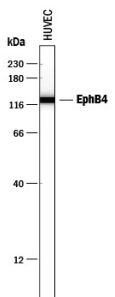
**Detection of EphB4 in MCF-7 Human Cell Line by Flow Cytometry.** MCF-7 human breast cancer cell line was stained with Goat Anti-Human EphB4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3038, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107). View our protocol for [Staining Membrane-associated Proteins](#).

**Immunohistochemistry**




**EphB4 in Human Kidney.** EphB4 was detected in immersion fixed paraffin-embedded sections of human kidney using 15 µg/mL Goat Anti-Human EphB4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3038) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

**Simple Western**



**Detection of Human EphB4 by Simple Western™.** Simple Western lane view shows lysates of HUVEC human umbilical vein endothelial cells, loaded at 0.2 mg/mL. A specific band was detected for EphB4 at approximately 127 kDa (as indicated) using 50 µg/mL of Goat Anti-Human EphB4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3038) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**BACKGROUND**

EphB4, also known as Htk, Myk1, Tyro11, and Mdk2, is a member of the Eph receptor tyrosine kinase family and binds Ephrin-B2. The A and B class Eph proteins have a common structural organization (1-4). The human EphB4 cDNA encodes a 987 amino acid (aa) precursor that includes a 15 aa signal sequence, a 524 aa extracellular domain (ECD), a 21 aa transmembrane segment, and a 427 aa cytoplasmic domain (5). The ECD contains an N-terminal globular domain, a cysteine-rich domain, and two fibronectin type III domains. The cytoplasmic domain contains a juxtamembrane motif with two tyrosine residues which are the major autophosphorylation sites, a kinase domain, and a conserved sterile alpha motif (SAM) (5). Activation of kinase activity occurs after membrane-bound or clustered ligand recognition and binding. The ECD of human EphB4 shares 89% aa sequence identity with mouse EphB4 and 42-45% aa sequence identity with human EphB1, 2, and 3. EphB4 is expressed preferentially on venous endothelial cells (EC) and inhibits cell-cell adhesion, chemotaxis, and angiogenesis. Opposing effects are induced by signaling through Ephrin-B2 expressed on arterial EC: adhesion, endothelial cell migration, and vessel sprouting (6). EphB4 signaling contributes to new vascularization by guiding venous EC away from Ephrin-B2 expressing EC. Ephrin-B2 signaling induces arterial EC to migrate towards nascent EphB4 expressing vessels (6). The combination of forward signaling through EphB4 and reverse signaling through Ephrin-B2 promotes *in vivo* mammary tumor growth and tumor-associated angiogenesis (7). EphB4 promotes the differentiation of megakaryocytic and erythroid progenitors but not granulocytic or monocytic progenitors (8, 9).

**References:**

1. Poliakov, A. *et al.* (2004) *Dev. Cell* **7**:465.
2. Surawska, H. *et al.* (2004) *Cytokine Growth Factor Rev.* **15**:419.
3. Pasquale, E.B. (2005) *Nat. Rev. Mol. Cell Biol.* **6**:462.
4. Davy, A. and P. Soriano (2005) *Dev. Dyn.* **232**:1.
5. Bennett, B.D. *et al.* (1994) *J. Biol. Chem.* **269**:14211.
6. Fuller, T. *et al.* (2003) *J. Cell Sci.* **116**:2461.
7. Noren, N.K. *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101**:5583.
8. Wang, Z. *et al.* (2002) *Blood* **99**:2740.
9. Inada, T. *et al.* (1997) *Blood* **89**:2757.