Human EphB4 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF3038

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human EphB4 in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant mouse (rm) EphB4 is observed and less than 1% cross-reactivity with recombinant human (rh) EphA4, rhEphA3, rhEphB2, and rhEphB3 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human EphB4 Leu16-Ala539 Accession # P54760
Formulation	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.

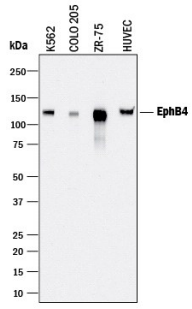
APPLICATIONS

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.*

	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	50 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Knockout Validated	EphB4 is specifically detected in HEK293T human embryonic kidney parental cell line but is not detectable in EphB4 knockout HEK293T cell line.	

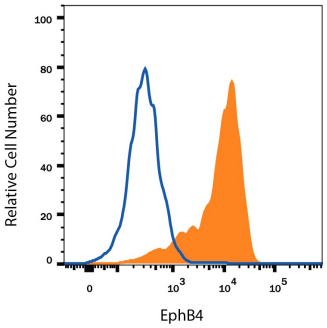
DATA

Western Blot



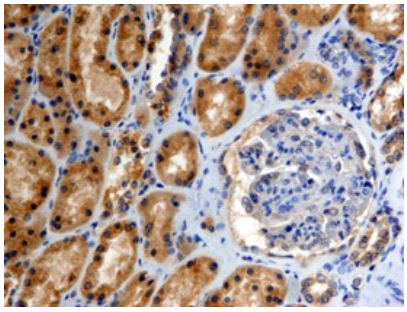
Detection of Human EphB4 by Western Blot. Western blot shows lysates of K562 human chronic myelogenous leukemia cell line, COLO 205 human colorectal adenocarcinoma cell line, ZR-75 human breast cancer cell line, and HUVEC human umbilical vein endothelial cells. PVDF membrane was probed with 2 µg/mL of 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



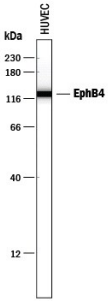
Detection of EphB4 in MCF-7 Human Cell Line by Flow Cytometry. MCF-7 human breast cancer cell line was stained with Goat Anti-Human EphB4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3038, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated 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.

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Human EphB4 Antibody

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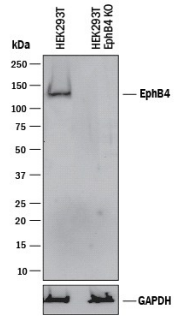
Knockout Validated



Western Blot Shows Human EphB4 Specificity by Using Knockout Cell Line. Western blot shows lysates of HEK293T human embryonic kidney parental cell line and EphB4 knockout HEK293T cell line (KO). 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 140 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in knockout HEK293T cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	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
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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 singaling 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:

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