Human/Mouse EphB2 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF467

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects mouse and human EphB2 in direct ELISAs and Western blots. In Western blots, approximately 5% cross-reactivity with recombinant rat (rr) EphB1, recombinant mouse (rm) EphA8, rmEphA6, rmEphB6, rmEphA3, rmEphA4, rmEphA7, rrEphA5, recombinant human EphA1, rmEphA2 and rmEphB3 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse EphB2 Val27-Lys548 Accession # P54763
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.

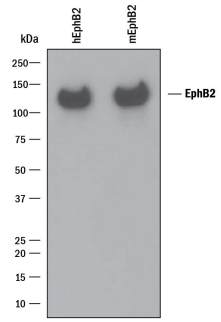
APPLICATIONS

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	0.1 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	1-15 µg/mL	See Below
Simple Western	1-20 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.	

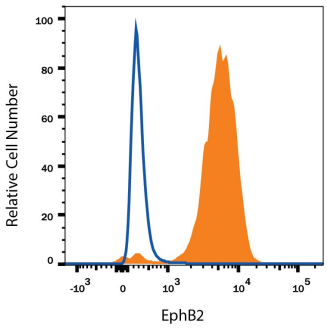
DATA

Western Blot



Detection of Recombinant Human and Mouse EphB2 by Western Blot. Western blot shows 25 ng of Recombinant Human EphB2 Fc Chimera (Catalog # Catalog # 5189-B2) and Recombinant Mouse EphB2 Fc Chimera (Catalog # Catalog # 467-B2). PVDF Membrane was probed with 0.1 µg/mL of Goat Anti-Human/Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF109). A specific band was detected for EphB2 at approximately 120 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.

Flow Cytometry



Detection of EphB2 in COLO 205 Human Cell Line by Flow Cytometry. COLO 205 human colorectal adenocarcinoma cell line was stained with Goat Anti-Human/Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467, filled histogram) or isotype control antibody (Catalog # Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.

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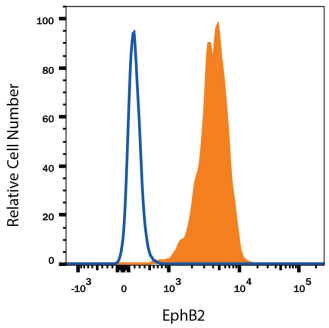
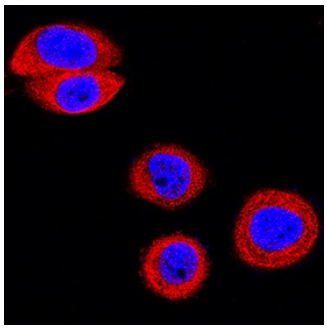
Flow Cytometry



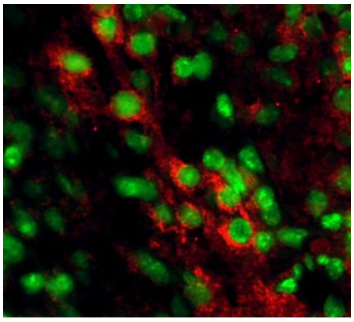
Detection of EphB2 in D3 Mouse Cell Line by Flow Cytometry. D3 mouse embryonic stem cell line was stained with Goat Anti-Human/Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467, filled histogram) or isotype control antibody (Catalog # Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.

Immunocytochemistry



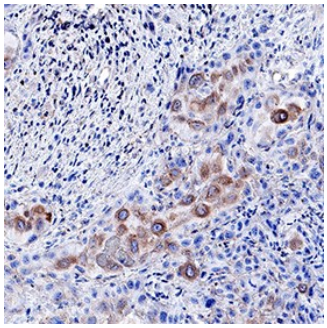
EphB2 in MBA-MB-468 Human Cell Line. EphB2 was detected in immersion fixed MBA-MB-468 human breast cancer cell line using Goat Anti-Human/Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



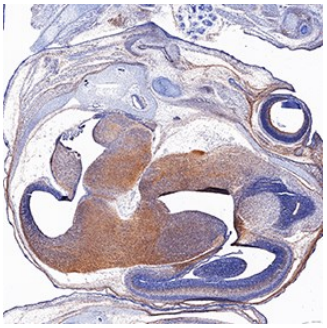
EphB2 in Embryonic Mouse Brain. EphB2 was detected in immersion fixed frozen sections of embryonic mouse brain (15 d.p.c.) using 15 µg/mL Goat Anti-Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467) overnight at 4 °C. Tissue was stained with the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # Catalog # NL001) and counterstained (green). View our protocol for Fluorescent IHC Staining of Frozen Tissue Sections.

Immunohistochemistry



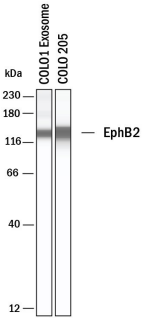
EphB2 in Human Esophageal Squamous Cell Carcinoma. EphB2 was detected in immersion fixed paraffin-embedded sections of human esophageal squamous cell carcinoma using Goat Anti-Human/Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in cancer cells. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry



EphB2 in Mouse Embryo. EphB2 was detected in immersion fixed frozen sections of mouse embryo (13 d.p.c.) using Goat Anti-Human/Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467) at 1.7 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to developing brain. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Simple Western



Detection of Human EphB2 by Simple Western™. Simple Western lane view shows lysates of Exosome Standards (COLO1) (Catalog # NBP2-49845) and COLO 205 human colorectal adenocarcinoma cell line, loaded at 0.5 mg/ml. A specific band was detected for EphB2 at approximately 135 kDa (as indicated) using 1 µg/ml of Goat Anti-Human/Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467) followed by HRP-conjugated Donkey Anti-Goat Secondary Antibody (Catalog # 042-206). This experiment was conducted under reducing conditions and using the 12-230kDa separation system.

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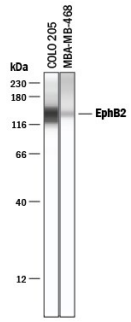
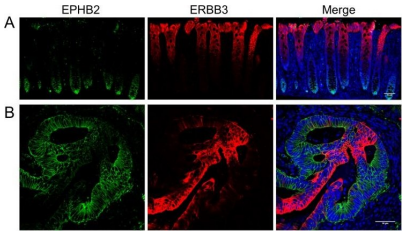
Simple Western



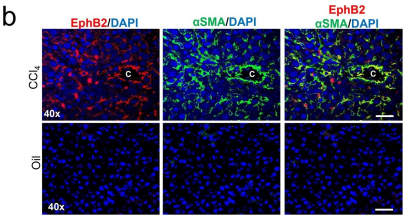
Detection of Human EphB2 by Simple Western™. Simple Western lane view shows lysates of COLO 205 human colorectal adenocarcinoma cell line and MBA-MB-468 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for EphB2 at approximately 139 and 146 kDa (as indicated) using 20 µg/mL of Goat Anti-Human/Mouse EphB2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF467) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Immunocytochemistry/ Immunofluorescence



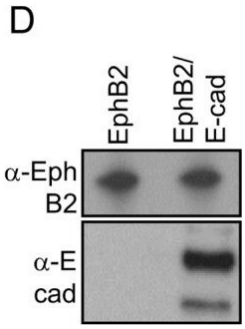
Detection of Human EphB2 by Immunocytochemistry/Immunofluorescence Colorectal tumours maintain tissue organisation similar to normal colon.Detection of EPHB2 (green) and ERBB3 (red, A and B) by co-immunofluorescence in normal colon (A) and colorectal cancer (B) (DAPI, blue). Scale bar, 50µm. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26367378), licensed under a CC-BY license. Not internally tested by R&D Systems.

Immunocytochemistry/ Immunofluorescence



Detection of Mouse EphB2 by Immunocytochemistry/Immunofluorescence Expression of EphB2 increases and is activated on HSCs after chronic CCl4 - induced liver injury. (a) Isolated cell fractions from livers of mice subjected to chronic CCl4 injections were analyzed for EphB2, Ephrin-B1, Ephrin-B2 and Ephrin-B3 mRNA levels using RT-qPCR. Results are shown as fold change compared to liver cell fractions obtained from vehicle-treated controls. Error bars represent mean ± SEM.; n = 6 animals; CD11b = macrophages, LSEC = Liver sinusoidal endothelial cells, HEP = Hepatocytes and HSCs = Hepatic stellate cells. (b) OCT liver sections from C57BL/6 J mice chronically injected with CCl4 or vehicle (oil) controls were stained with EphB2 (red), αSMA (green) and DAPI/DNA (blue) and analyzed using confocal microscopy. Scale bar = 100 µm, "C" denotes the central vein. All images are representative of 5 mice per group. (c) OCT liver sections from C57BL/6 J mice chronically injected with CCl4 or vehicle controls were stained with phospho-EphB1/EphB2-Y594 (red), PDGFRβ (green) and DAPI/DNA (blue) and analyzed using confocal microscopy. Scale bar = 50 µm. All images are representative of 5 mice per group. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29416088), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



Detection of Human EphB2 by Western Blot Increased cell-cell adhesion within one cell population is required for the formation of tightly packed cell clusters.A) Simulation of cell-cell segregation using the same adhesion term in both cell types (Aeph = Aephrin = 100, left panel) vs. increased adhesion only in the green cell population (Aeph =110, Aephrin =100, right panel). Both simulations started with the same number of Eph (green) and ephrin (black) expressing cells. In the "Equal adhesion" case, an 'Islands-in-a-sea' pattern is less apparent. B) Representative images from segregation assays of unlabelled ephrin-B1 cells co-cultured with Cell Tracker-green labelled (green staining) EphB2 cells, without (left) or with E-cadherin-cherry expression (red staining, right); scale bar, 75 µm. C) Quantitation of cell densities in the cell clusters shown in B (n= 10). D) Western blot analysis of lysates from parental and E-cadherin-cherry-transduced cells, using the indicated antibodies. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0111803), licensed under a CC-BY license. Not internally tested by R&D Systems.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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

EphB2, also known as Cek5, Nuk, Erk, Qek2, Tyro5, Sek3, Hek5, and Drt (1), is a member of the Eph receptor family which binds members of the ephrin ligand family. There are two classes of receptors, designated A and B. Both the A and B class receptors have an extracellular region consisting of a globular domain, a cysteine-rich domain, and two fibronectin type III domains. This is followed by the transmembrane region and the cytoplasmic region. The cytoplasmic region contains a juxtamembrane motif with two tyrosine residues which are the major autophosphorylation sites, a kinase domain, and a conserved sterile alpha motif (SAM) in the carboxy tail which contains one conserved tyrosine residue. Activation of kinase activity occurs after ligand recognition and binding. EphB2 has been shown to bind ephrin-B1, ephrin-B2, and ephrin-B3 (2, 3). The extracellular domains of human and mouse EphB2 share 99% amino acid identity. Only membrane-bound or Fc-clustered ligands are capable of activating the receptor *in vitro*. Soluble monomeric ligands bind the receptor but do not induce receptor autophosphorylation and activation (2). *In vivo*, the ligands and receptors display reciprocal expression (3). It has been found that nearly all the receptors and ligands are expressed in developing and adult neural tissue (3). The ephrin/Eph families also appear to play a role in angiogenesis (3).

References:

1. *Eph Nomenclature Committee [letter]* (1997) Cell **90:**403.
2. Flanagan, J.G. and P. Vanderhaeghen (1998) Annu. Rev. Neurosci. **21**:309.
3. Pasquale, E.B. (1997) Curr. Opin. Cell Biol. **9:**608.

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