Human/Rat EphA5 Antibody

Monoclonal Mouse IgG_1 Clone # 86731

Catalog Number: MAB541

DESCRIPTION

Species Reactivity	Human/Rat
Specificity	Detects rat EphA5 in direct ELISAs and Western blots. In direct ELISAs, 5-15% cross reactivity with recombinant mouse (rm) EphA4 and rmEpha8 is observed and no cross-reactivity with recombinant human (rh) EphA1, rmEphA2, -A3, -A6, -A7, recombinant rat EphB1, -B2, or rmEphB3 is observed.
Source	Monoclonal Mouse IgG_1 Clone # 86731
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant rat EphA5 Ser58-Gln573 (Asp170Glu) Accession # P54757
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 as a 0.2 μm filtered solution in PBS.

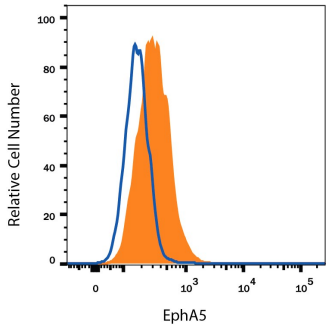
APPLICATIONS

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	1 µg/mL	Recombinant Rat EphA5 Fc Chimera (Catalog # 541-A5)
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	8-25 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.	

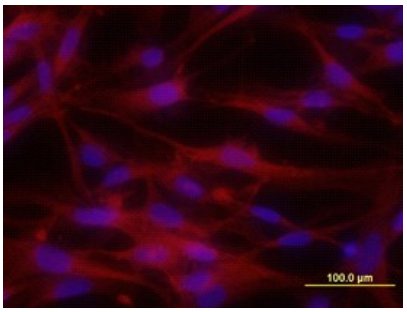
DATA

Flow Cytometry



Detection of EphA5 in U-118-MG Human Cell Line by Flow Cytometry. U-118-MG human glioblastoma/astrocytoma cell line was stained with Mouse Anti-Human/Rat EphA5 Monoclonal Antibody (Catalog # MAB541, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). View our protocol for Staining Membrane-associated Proteins.

Immunocytochemistry



EphA5 in U-118-MG Human Cell Line. EphA5 was detected in immersion fixed U-118-MG human glioblastoma/astrocytoma cell line using 10 µg/mL Mouse Anti-Human/Rat EphA5 Monoclonal Antibody (Catalog # MAB541) for 3 hours at room temperature. Cells were stained with the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counter-stained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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.

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BACKGROUND

EphA5, also known as Ehk1, Bsk, Cek7, Hek7, and Rek7 (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 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. EphA5 has been shown to bind ephrin-A5, ephrin-A1, ephrin-A2, ephrin-A3, and ephrin-A4 (2, 3). The extracellular domains of rat EphA5 share 98.5% amino acid identity with the mouse homolog and 96.5% identity with the human homolog. Only membrane-bound or Fc-clustered ligands are capable of activating the receptor *in vitro*. While soluble monomeric ligands bind the receptor, they 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 receptors and ligands are expressed in developing and adult neural tissue (3). The Eph/ephrin 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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