

Product Information

EphB1/Fc Chimera

Rat, Recombinant

Expressed in NSO mouse myeloma cells

Product Number **E 9277**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

Synonyms: EphB1; Cek6; Hek6; Net; Elk

Product Description

Recombinant Rat EphB1/Fc Chimera is a DNA sequence encoding the signal peptide from human CD33, joined with the extracellular domain of mature rat EphB1 (amino acid residues 18-538)¹ that was fused to the Fc portion of human IgG1 via a peptide linker. The chimeric protein is expressed in a mouse myeloma cell line, NSO. Recombinant EphB1 is a disulfide-linked homodimer. The amino-terminus is Met(18) determined by N-terminal sequencing. The calculated molecular mass of the reduced protein is ~85 kDa, but as a result of glycosylation, the recombinant protein migrates as an ~102 kDa protein in SDS-PAGE under reducing conditions.

The Eph receptor family, of which EphB1 is a member, binds members of the Ephrin ligand family. Two classes of receptors exist, designated A and B, that have an extracellular domain made up of a globular domain, a cysteine-rich domain, and two fibronectin type III domains, followed by the transmembrane region and cytoplasmic region. The cytoplasmic region is a juxtamembrane region with two tyrosines, the major autophosphorylation sites, along with a kinase domain, and a conserved sterile α -motif (SAM) in the carboxy-terminus, the latter including one conserved tyrosine. Ligand recognition and binding leads to activation of the intrinsic kinase activity. EphB1 binds to Ephrin-B1, Ephrin-B2, Ephrin-A1, Ephrin-A3, Ephrin-A4 and Ephrin-B3.^{2,3} Human and mouse EphB1 extracellular domains share approximately 99% homology. Only membrane-bound or Fc-clustered ligands have been shown to

activate the receptor *in vitro*. Soluble monomeric ligands can bind to the receptor, but do not induce receptor autophosphorylation and activation.² The ligands and receptors display reciprocal expression *in vivo*.³

Nearly all Ephrin-related receptors and ligands have been found to be expressed in developing and adult neural tissue.³ The Eph/Ephrin families may also play a role in angiogenesis.³

Reagent

The product is supplied as ~200 μg of protein lyophilized from a 0.2 μm filtered solution in 20 mM Tris, pH 8.5.

Preparation Instructions

Reconstitute the vial contents with sterile PBS. Stock solution concentration should be no less than 100 $\mu\text{g}/\text{mL}$. The carrier-free protein should be used immediately upon reconstitution to avoid losses in activity due to non-specific binding to the inside surface of the vial. For long term storage as a dilute solution, a carrier protein (e.g. 0.1% HSA or BSA) should be added to the vial.

Storage/Stability

Lyophilized samples are stable for up to twelve months from date of receipt at $-20\text{ }^{\circ}\text{C}$ to $-70\text{ }^{\circ}\text{C}$. Upon reconstitution in the presence of a carrier protein, this product can be stored under sterile condition at 2-8 $^{\circ}\text{C}$ for up to one month. For extended storage, store in working aliquots at $-20\text{ }^{\circ}\text{C}$ or $-70\text{ }^{\circ}\text{C}$. Repeated freeze-thaw cycles should be avoided. Do not store in "frost-free" freezer.

Product Profile

The activity is measured by the ability of immobilized recombinant rat EphB1/Fc (2 µg/mL, 100 µL/well) to bind recombinant mouse Ephrin-B1/Fc in a functional ELISA assay.

Optimal dilutions should be determined by each laboratory for each application.

Purity: >95% by SDS-PAGE, visualized by silver stain.

Endotoxin level: < 1.0 endotoxin units (EU)/µg of protein as determined by the LAL (Limulus amoebocyte lysate) method.

References

1. Lhoták, V., Characterization of elk, a brain-specific receptor tyrosine kinase. *Mol. Cell. Biol.*, **11**, 2496-2402 (1991).
2. Flanagan, J.G. and P. Vanderhaegen, The ephrins and Eph receptors in neural development. *Annu. Rev. Neurosci.*, **21**, 309-345 (1998).
3. Pasquale, E.B., The Eph family of receptors. *Curr. Opin. Cell Biol.*, **9**, 608-615 (1997).

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