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Product Information

EphA8/Fc Chimera

Recombinant Mouse
Expressed in NSO cells

Product Number **E 8777**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

Synonyms: Eek and Hek3

Product Description

Recombinant Mouse EphA8/Fc Chimera consists of amino acid residues 1-540 of the extracellular domain of mouse EphA8¹ fused by means of a polypeptide linker to the Fc portion of human IgG₁ that is histidine-tagged at the carboxyl terminus. The chimeric protein is expressed in a mouse myeloma cell line, NSO. Recombinant EphA8 is a disulfide-linked homodimer. The amino terminus is Gly(27) based on N-terminal sequencing. The calculated molecular mass of the reduced protein is approximately 84.2 kDa, but as a result of glycosylation, the recombinant EphA8/Fc chimera migrates as an approximately 100-120 kDa protein on reducing SDS-PAGE.

The Eph receptor family, of which EphA8 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 alpha motif (SAM) in the carboxyl terminus, the latter including one conserved tyrosine. Ligand recognition and binding leads to kinase activation of the intrinsic kinase activity. EphA8 binds to ephrin-A2, ephrin-A3, and ephrin-A5.^{2,3} Full length human and rat homologs have not been successfully cloned. Only membrane-bound or Fc-clustered ligands have been shown to activate the receptor *in vitro*. Soluble monomeric ligands can bind 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

Recombinant Mouse EphA8/Fc Chimera is supplied as approximately 200 μg of protein lyophilized from a sterile filtered solution in 20 mM Tris, pH 8.0.

Preparation Instructions

Reconstitute the vial contents with sterile phosphate buffered saline. Stock solution concentration should be no less than 100 $\mu\text{g}/\text{ml}$.

Storage/Stability

Store lyophilized samples at $-20\text{ }^{\circ}\text{C}$. Upon reconstitution, store at $2-8\text{ }^{\circ}\text{C}$ for up to one month. For extended storage, store in working aliquots at $-20\text{ }^{\circ}\text{C}$. Repeated freeze-thaw cycles should be avoided. Do not store in a frost-free freezer.

Product Profile

EphA8/Fc activity is measured by its ability to bind immobilized recombinant human Ephrin-A5/Fc in a functional ELISA assay. Immobilized recombinant mouse EphA8/Fc (2 $\mu\text{g}/\text{ml}$, 100 $\mu\text{l}/\text{well}$) binds recombinant human Ephrin-A5/Fc with a linear range of 0.31-20 ng/ml.

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

Purity: > 90% by SDS-PAGE, visualized by silver stain.

Endotoxin level: < 0.1 ng/ μg of protein as determined by the LAL (Limulus amoebocyte lysate) method.

References

1. Park, S., and M.P. Sanchez, The Eek receptor, a member of the Eph family of tyrosine protein kinases, can be activated by three different Eph ligands, *Oncogene*, **14**, 533-542 (1997).
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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