

## Product Information

### EphA4 EXTRACELLULAR DOMAIN/Fc CHIMERA

Mouse, Recombinant

Expressed in NSO mouse myeloma cells

Product Number **E 8277**

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

Synonyms: Sek, Sek1, Cek8, Hek8, Tyro1

#### Product Description

Recombinant mouse EphA4 extracellular domain/Fc chimera consists of amino acid residues 1-547 (extracellular domain of mouse EphA4)<sup>1</sup> that was fused by means of a polypeptide linker to the Fc portion of human IgG<sub>1</sub> that is histidine-tagged at the carboxyl terminus. The chimeric protein is expressed in a mouse myeloma cell line, NSO. Recombinant EphA4 is a disulfide-linked homodimer. The amino terminus is Val(20) based on N-terminal sequencing. The calculated molecular mass of the reduced protein is approximately 85.7kDa, but as a result of glycosylation, the recombinant EphA4/Fc chimera migrates as an approximately 110 kDa protein on reducing SDS-PAGE.

The Eph receptor family, of which EphA4 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 activation of the intrinsic kinase activity. EphA4 binds to Ephrin-A1, Ephrin-A2, Ephrin-B2, Ephrin-A3, Ephrin-B3, Ephrin-A4, and Ephrin-A5.<sup>2,3</sup> Human and mouse EphA4 extracellular domains share approximately 95% homology. 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.<sup>2</sup> The ligands and receptors display reciprocal expression *in vivo*.<sup>3</sup>

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

#### Reagents

Recombinant mouse EphA4 extracellular domain/Fc chimera is supplied as approximately 200  $\mu\text{g}$  of protein lyophilized from a sterile filtered phosphate-buffered saline (PBS) solution.

#### Preparation Instructions

Reconstitute the vial contents with sterile PBS.

Stock solution concentration should be no less than 100  $\mu\text{g}/\text{ml}$ .

#### Storage/Stability

Lyophilized samples are stable for more than six months at  $-20^{\circ}\text{C}$ . Upon reconstitution, store at  $2-4^{\circ}\text{C}$  for up to one month. For extended storage, store in working aliquots at  $-20^{\circ}\text{C}$ . Repeated freeze-thaw cycles should be avoided. Do not store in a frost-free freezer.

#### Product Profile

EphA4/Fc activity is measured by its ability to bind immobilized recombinant mouse Ephrin-A5/Fc in a functional ELISA assay. Immobilized recombinant mouse EphA4/Fc (2  $\mu\text{g}/\text{ml}$ , 100  $\mu\text{l}/\text{well}$ ) binds recombinant mouse Ephrin-A5/Fc with a linear range of 0.16–10 ng/ml. Optimal dilutions should be determined by each laboratory for each application.

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

Endotoxin level: < 0.1 ng/ $\mu\text{g}$  of protein as determined by the LAL (Limulus amoebocyte lysate) method.

## References

1. Gilardi-Hebenstreit, P. *et al.*, An Eph-related receptor protein tyrosine kinase gene segmentally expressed in the developing mouse hindbrain. *Oncogene*, **7**, 2499-2506 (1992).
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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