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# **ProductInformation**

## EphA3 EXTRACELLULAR DOMAIN/Fc CHIMERA

Mouse, Recombinant
Expressed in NSO mouse myeloma cells

Product Number **E 8152** Storage Temperature –20 °C

Synonyms: Cek4, Mek4, Hek, Tyro4, Hek4

# **Product Description**

Recombinant mouse EphA3 extracellular domain/Fc chimera consists of amino acid residues 1-541 (extracellular domain of mouse EphA3)¹ that was 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 EphA3 is a disulfide-linked homodimer. The amino terminus is Glu(21) based on N-terminal sequencing. The calculated molecular mass of the reduced protein is approximately 86 kDa, but as a result of glycosylation, the recombinant EphA3/Fc chimera migrates as an approximately 110 kDa protein on reducing SDS-PAGE.

The Eph receptor family, of which EphA3 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. EphA3 binds to Ephrin-A1, Ephrin-B1, Ephrin-A2, Ephrin-A3, Ephrin-A4, and Ephrin-A5. 2,3 Human and mouse EphA3 extracellular domains share approximately 96% homology. Only membranebound 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.3

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 EphA3 extracellular domain/Fc chimera is supplied as approximately 200 µg 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 µg/ml.

## Storage/Stability

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

### **Product Profile**

EphA3/Fc activity is measured by its ability to bind immobilized recombinant mouse Ephrin-A5/Fc in a functional ELISA assay. Immobilized recombinant mouse EphA3/Fc (2  $\mu$ g/ml, 100  $\mu$ l/well) binds recombinant mouse Ephrin-A5/Fc with a linear range of 0.078–5.0 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 amebocyte lysate) method.

#### References

- 1. Sajjadi, F.G. *et al*, Identification of a new eph-related receptor tyrosine kinase gene from mouse and chicken that is developmentally regulated and encodes at least two forms of the receptor. New Biol., **3**, 769-778 (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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