

## Product Information

### EphB2 EXTRACELLULAR DOMAIN/Fc CHIMERA

Mouse, Recombinant

Expressed in NSO mouse myeloma cells

Product Number **E 9402**

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

Synonyms: Cek5; Hek5; Drt; Tyro5; Sek3; Qek2; Nuk; Erk<sup>5</sup>

#### Product Description

Recombinant mouse EphB2 extracellular domain/Fc chimera consists of amino acid residues 1-548 (extracellular domain of mouse EphB2)<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 EphB2 is a disulfide-linked homodimer. The amino terminus is Val(27) based on N-terminal sequencing. The calculated molecular mass of the reduced protein is approximately 85.3 kDa, but as a result of glycosylation, the recombinant EphB2/Fc migrates as a 100–110 kDa protein on reducing SDS-PAGE.

The Eph receptor family, of which EphB2 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. EphB2 binds to Ephrin-B1, Ephrin-B2, and Ephrin-B3.<sup>2,3</sup> Human and mouse EphB2 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 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 EphB2 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  $-20\text{ }^{\circ}\text{C}$ . Upon reconstitution, store at  $2-4\text{ }^{\circ}\text{C}$  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

EphB2/Fc activity is measured by its ability to bind immobilized recombinant mouse Ephrin-B2/Fc in a functional ELISA assay. Immobilized recombinant mouse EphB2/Fc (2  $\mu\text{g}/\text{ml}$ , 100  $\mu\text{l}/\text{well}$ ) binds recombinant mouse Ephrin-B2/Fc with a linear range of 0.039 – 2.5 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/ $\mu\text{g}$  of protein as determined by the LAL (Limulus amoebocyte lysate) method.

## References

1. Henkemeyer, M., et al., Immunolocalization of the Nuk receptor tyrosine kinase suggests roles in segmental patterning of the brain and axonogenesis. *Oncogene*, **9**, 1001-1014 (1994).
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)
4. Ciossek, T., et al., Cloning, characterization, and differential expression of MDK2 and MDK5, two novel receptor tyrosine kinases of the eck/eph family. *Oncogene*, **11**, 2085-2095 (1995).
5. Unified nomenclature for Eph family receptors and their ligands, the ephrins. Eph Nomenclature Committee [letter]. *Cell*, 90(3):403 – 404 (1997).

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