

Product Information

EPHRIN-A5 EXTRACELLULAR DOMAIN/FC CHIMERA

Human, Recombinant
Expressed in NSO mouse myeloma cells

Product Number **E 0528**
Storage Temperature -20°C

Synonyms: LERK-7; EFL-5; RAGS; AL-1

Product Description

Recombinant human Ephrin-A5 extracellular domain/Fc chimera consists of amino acid residues 1 - 203 (extracellular domain of human Ephrin-A5)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 Ephrin A5 is a disulfide-linked homodimer. The amino terminus is Gln(21) determined by N-terminal sequencing. The calculated molecular mass of the reduced protein is approximately 48.6 kDa, but as a result of glycosylation, the recombinant Ephrin-A5/Fc chimera migrates as a 50 - 55 kDa protein on reducing SDS-PAGE.

The Ephrin ligand family, of which Ephrin-A5 is a member, binds members of the Eph receptor family. All ligands share a conserved extracellular sequence, thought to correspond to the receptor binding domain. The conserved sequence contains approximately 125 amino acids including four invariant cysteines. A-class ligands have a GPI anchor after the conserved sequence. Ephrin-A5 can bind EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, and EphB1.^{2,3} Human and mouse Ephrin-A5 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.² The ligands and receptors display reciprocal expression *in vivo*.³

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

Reagents

Recombinant human Ephrin-A5 extracellular domain/Fc chimera is 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 $\mu\text{g/ml}$.

Storage/Stability

Lyophilized samples are stable for at least six months at -20°C . Upon reconstitution, store at $2-4^{\circ}\text{C}$ 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 frost-free freezer.

Product Profile

Ephrin-A5/Fc activity is measured by its ability to compete with biotinylated human Ephrin-A5/Fc chimera for binding to immobilized recombinant mouse EphA3/Fc in a functional ELISA assay. Approximately 0.4-2 $\mu\text{g/ml}$ of human Ephrin-A5/Fc chimera will reduce by 50% the binding of 10 ng/ml biotinylated human Ephrin-A5/Fc chimera to immobilized recombinant mouse EphA3/Fc (2 $\mu\text{g/ml}$, 100 $\mu\text{l/well}$). 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. Winslow, J.W. *et al*, Cloning of AL-1, a ligand for an Eph-related tyrosine kinase receptor involved in axon bundle formation. *Neuron*, **14**, 973-981 (1995).
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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