

Product Information

Notch-3/Fc Chimera

Human, Recombinant
Expressed in *Sf21* cells

Product Number **N 9036**

Product Description

Recombinant Human Notch-3/Fc Chimera is produced from a DNA sequence encoding the first eleven EGF repeats of the extracellular domain of human Notch-3 (amino acid residues Ala 40-Glu 467),¹ fused to the signal peptide of human CD33 at the amino-terminus and to the Fc region of human IgG1 at the carboxy-terminus. The chimeric protein is expressed in a stable insect cell line, *Sf21*. Recombinant human Notch-3/Fc chimera, generated by the proteolytic removal of the signal peptide, is a disulfide-linked homodimeric protein. Based on N-terminal sequencing, mature Notch-3/Fc starts at Ala 40. The calculated molecular mass is 71.4 kDa. As a result of glycosylation, the recombinant protein migrates as an 85-95 kDa protein in SDS-PAGE under reducing conditions. Human Notch3 and mouse Notch3 show 90% amino acid sequence identity.

Human Notch-3 is a member of Notch family of type I transmembrane glycoproteins involved in a number of early-event developmental processes.² Notch signaling is important for specifying cell fates and for defining boundaries between different cell types. The molecule is synthesized as a 2321 amino acid precursor that contains a 39 amino acid signal sequence, a 1603 amino acid extracellular region, a 21 amino acid transmembrane segment, and a 658 amino acid cytoplasmic domain. The large Notch extracellular domain has 34 EGF-like repeats followed by three notch/Lin-12 repeats (LNR).³ Of the 34 EGF-like repeats, the 11th and 12th are necessary and sufficient for binding the ligands Serrate and Delta in *Drosophila*.⁴ The extracellular domain of Notch receptors interacts with the extracellular domain of transmembrane ligands Jagged, Delta, and Serrate.

In mammals, four Notch genes have been identified (Notch1-4) that are expressed in a wide variety of cells and play a crucial role in differentiation and development.⁵⁻⁷ The Notch protein family is a group of highly conserved proteins important in the determination of cell fate and maintenance of progenitors in many developmental systems. This family of proteins function both as membrane cell receptors and as transcription factors.

Activation of Notch by cell-cell interactions causes a transcription inhibitory effect that enables inhibition of differentiation in some cells but not in others. As a consequence, some cells adopt a particular fate while other progenitors remain uncommitted. The Notch protein is important in cell fate during myogenesis, neurogenesis, oogenesis, and wing and eye development in *Drosophila*.

In humans, mutations in Notch3 cause an autosomal dominant condition called CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). This condition is characterized by recurrent ischemic strokes at an early age without any underlying vascular risk and progressive dementia. The mutations leading to CADASIL are clustered in the first 5 EGF repeats of the Notch3 gene.⁸

Reagent

Recombinant Human Notch-3/Fc Chimera is supplied as approximately 50 µg of protein lyophilized from a 0.2 µm filtered solution in phosphate buffered saline.

Storage/Stability

Prior to reconstitution, store at -20 °C. The reconstituted product (in the presence of a carrier protein) may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots. Avoid repeated freezing and thawing.

Preparation Instructions

Reconstitute the contents of the vial using 0.2 µm filtered phosphate buffered saline. Prepare a stock solution of no less than 100 µg/ml. The carrier-free protein should be used immediately upon reconstitution to avoid losses in activity due to non-specific binding to the inside surface of the vial. For long term storage as a dilute solution, a carrier protein such as 0.1% human serum albumin or bovine serum albumin should be added to the vial.

Product Profile

The biological activity of human Notch-3/Fc Chimera is measured by its ability to bind Jagged-1. Immobilized recombinant human Notch-3/Fc at 5 µg/ml (100 µl/well) can bind recombinant rat Jagged-1/Fc with a linear range of 8-500 ng/ml in an ELISA.

Endotoxin: < 1.0 EU (endotoxin unit)/µg cytokine as determined by the LAL method.

References

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