SYMANSIS
CELL SIGNALING SCIENCE

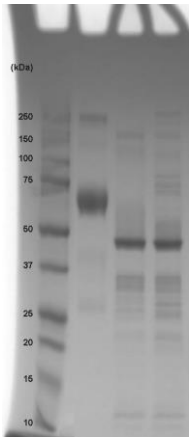
Product Data Sheet

human cell expressed IL-4 R alpha (207aa)-Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human IL-4 R alpha (aa 1-232) was fused to the Fc region of human IgG1 (aa 90-330). The chimeric protein was expressed in modified human 293 cells.
Molecular Mass	Under reducing conditions Symansis IL-4 R alpha (207aa) – Fc^{HCX} Chimera migrates as a broad band between 60 and 75 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified IL-4 R alpha (207aa) - Fc Chimera that has a predicted monomeric molecular mass of 51 kDa.
pI	Symansis IL-4 R alpha (207aa) – Fc^{HCX} Chimera separates into a number of isoforms with a pI between 5.5 and 6.5 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IL-4 R alpha (207aa) – Fc Chimera that has a predicted pI of 6.5.
% Carbohydrate	Symansis purified IL-4 R alpha (207aa) – Fc^{HCX} Chimera consists of 15-35% carbohydrate by weight.
Glycosylation	Symansis IL-4 R alpha (207aa) – Fc^{HCX} Chimera contains N-linked oligosaccharides and may have O-linked oligosaccharides.
Purity	>95%, as determined by SDS-PAGE and visualized by silver stain.
Formulation	When reconstituted in 0.5 ml sterile phosphate-buffered saline, the solution will contain 1% human serum albumin (HSA) and 10% trehalose.
Reconstitution	It is recommended that 0.5 ml of sterile phosphate-buffered saline be added to the vial.
Storage	Lyophilized products should be stored at 2 to 8°C. Following reconstitution short-term storage at 4°C is recommended and longer-term storage of aliquots at -18 to -20°C. Repeated freeze thawing is not recommended.
Activity	The ED_{50} of IL-4 R alpha (207aa) – Fc^{HCX} Chimera is typically 45-60 ng/ml as measured by its ability to neutralize IL-4 mediated proliferation of the human growth-factor dependent TF-1 cell line.
Background Information	The IL-4 receptor is expressed on a wide variety of cells, including T and B cells monocytes, granulocytes, fibroblasts, epithelial and endothelial cells. The IL-4 receptor comprises the IL-4 R alpha (IL-4Ra) subunit and the common gamma chain (shared with other cytokine receptors, including IL-2, IL-7, Il-9 and IL-15). The effects of IL-4 are mediated through the IL-4 receptor (IL-4R). IL-4Ra can also bind the Interleukin-13 receptor-alpha sub-unit to form an alternate receptor, allowing IL-13 to elicit a subset of the biological activities of IL-4. The IL-4/IL-13 response is important for promoting differentiation of the Th2 subtype of T-cells involved in allergic responses and inflammation. These cytokines can also act by stimulating production of IgE, the IgE receptor, chemokine and mucous at the site of allergic inflammation. Symansis IL-4 R alpha is produced as an ECD-Fc fusion protein with the aim of enhancing its activity. ECD-Fc fusion proteins have an advantage over soluble receptors because many receptors are only functional in dimeric form. Fusion to the Fc domain of IgG1 induces dimerization due to the ability of the Fc domain to form disulfide bonds. The resulting dimeric receptor ECD-Fc mimics the activated form of the receptor and possesses enhanced affinity for its cognate ligand relative to its monomeric form. For a review of the signalling mechanisms and biological functions of IL-4 receptor please see Nelms *et al.* (1999) Annu Rev Immunol 17:701-738.

human cell expressed IL-4 R alpha (207aa)-Fc^{HCX} Chimera

1D gel



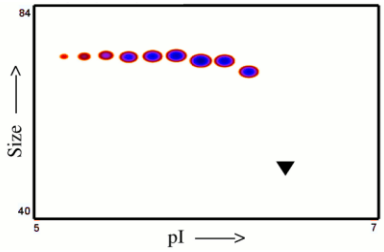
1D gel data

Lane 1 – MW markers; Lane 2 – IL-4 R alpha (207aa) – Fc^{HCX} Chimera; Lane 3 – IL-4 R alpha (207aa) – Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – IL-4 R alpha (207aa) – Fc^{HCX} Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans. Approximately 5 µg of protein was loaded per lane; Gel was stained using Coomassie.

Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

Densitometry

Post-translational modifications result in protein heterogeneity. The densitometry scan demonstrates the purified human cell expressed protein exists in multiple isoforms, which differ according to their level of post-translational modification. Expression of these isoforms is highly significant for cell biology, as they more closely resemble the native human proteins.



The triangle indicates theoretical pI and MW of the protein. The original 2D gel from which the densitometry scan was derived is shown above.

Theoretical Sequence

MKVLQEPTCVSDYMSISTCEWKMNGPTNCSTELRLLYQLVFLLSEAHTCIPEN
NGGAGCVCHLLMDDVVSADNYTLDLWAGQQLLWKGSFKPSEHVKPRAPGNL
TVHTNVSDTLLLTWSNPYPPDNYLYNHLTYAVNIWSENDPADFRIYNVTYLEP
SLRIAASTLKSGISYRARVRAWAQCYNTTWSEWSPSTKWHNSYREPFEQHG
SSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK