SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed IL-3 R alpha-Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human Interleukin 3 receptor alpha chain (aa 1-18) was fused to the Fc region of human IgG1 (aa 93-330). The chimeric protein was expressed in modified human 293 cells.
Molecular Mass	Symansis IL-3 R alpha-Fc^{HCX} Chimera migrates as a broad band between 85 and 100 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified IL-3 R alpha-Fc that has a predicted molecular mass of 60.6 kDa.
pI	Symansis IL-3 R alpha-Fc^{HCX} Chimera separates into a number of isoforms with a pI between 6.2 and 9.0 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IL-3 R alpha-Fc that has a predicted pI of 8.43.
% Carbohydrate	Symansis purified IL-3 R alpha-Fc^{HCX} Chimera consists of 25-40% carbohydrate by weight.
Glycosylation	Symansis IL-3 R alpha-Fc^{HCX} Chimera has N-linked and possibly 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.
Background Information	Interleukin-3 (IL-3) is a pleiotropic cytokine that regulates the proliferation, maturation, and survival of progenitor cells of the myeloid, erythroid, and megakaryocyte lineage. The biological activity of IL-3 is exerted through the IL-3 receptor (IL-3R) complex. The IL-3R complex is a heterodimer comprising IL-3 R alpha (IL-3Ra) and the common beta chain, which also involved in IL-5 and GM-CSF receptor activity. IL-3Ra is constitutively expressed on hematopoietic progenitor (CD34+) cells and mediates their proliferation and differentiation. It is also found on monocytes, neutrophils, basophils, eosinophils, megakaryocyte and erythroid precursors, mast cells, macrophages, a subpopulation of CD5+B-lymphocytes and some endothelial cells. Deregulated IL-3R signaling has been implicated in a number of disease states such as myeloid leukemia's, follicular B-cell lymphoma, allergies and B-cell acute lymphoblastic leukemia (ALL). IL-3 R alpha is a type I transmembrane glycoprotein and contains 6 potential N-linked glycosylation sites. For a review on cytokine signal transduction for the IL-3/IL-5/GM-CSF receptor family please refer to Geijsen *et al*., (2001) *Cytokine Growth Factor Rev.* **12**(1): 19-25.

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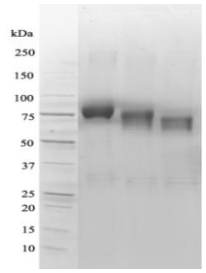
1D gel



2D gel

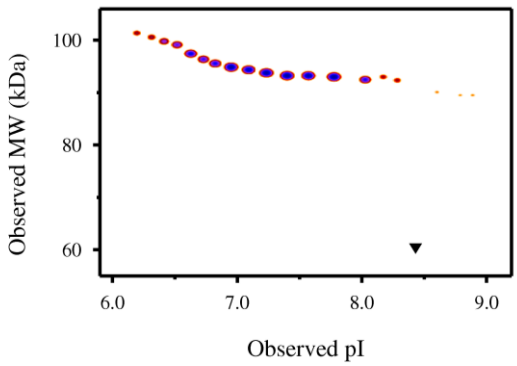
1D gel data

Lane 1 – MW markers; Lane 2 – IL-3 R alpha-Fc^{HCX} Chimera; Lane 3 - IL-3 R alpha-Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – IL-3 R alpha-Fc^{HCX} Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 10 µg of protein loaded per lane; Deep Purple™ stained. Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Subsequent drop in MW after treatment with glycosidase cocktail indicates presence of O-linked glycans. Faint bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of IL-3 R alpha-Fc^{HCX} Chimera without carrier protein was reduced and alkylated and focused on a 3-10 IPG strip then run on a 4-20% Tris-HCl 2D gel. 40 µg of protein loaded per lane; Deep Purple™ stained. Spot train indicates presence of multiple isoforms of IL-3 R alpha-Fc^{HCX} Chimera. Spots within the spot train were cut from the gel and identified as IL-3 R alpha-Fc^{HCX} Chimera by protein mass fingerprinting. Experimental details and results are available upon request.

Densitometry



Post-translational modifications result in protein heterogeneity. The densitometry scan, derived from the 2D gel image above, 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.

Theoretical Sequence

TKEDPNPPITNLRMKAKAQQLTWDLNRNVTDIECVKDADYSMPAVNNSYCQFGAISLCEVTNY
TVRVANPPFSTWILFPENSGKPWAGAENLTCWIHDVDFLSCSWAVGPGAPADVQYDLYLNVA
NRRQQYECLHYKTDAQGTRIGCRFDDISRLSSGSQSSHILVRGRSAAFGIPCTDKFVVFSQIEI
LTPPNMTAKCNKTHSFMHWKMRSHFNRKFRYELQIQKRMQPVITEQVRDRTSFQLLNPGTYT
VQIRARERVYEFLSAWSTPQRFECDQEEGANTRGGRVDGIQWIPKVDKKVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCRVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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