SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed IL-10 R alpha-Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human Interleukin 10 receptor alpha (IL-10 R alpha) (aa 1-232) 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-10 R alpha-Fc^{HCX} Chimera migrates as a broad band between 55 and 85 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified IL-10Ra-Fc that has a predicted molecular mass of 51.1 kDa.
pI	Symansis IL-10 R alpha-Fc^{HCX} Chimera separates into a number of isoforms with a pI between 5.7 and 8.5 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IL-10 R alpha-Fc that has a predicted pI of 7.69.
% Carbohydrate	Symansis purified IL-10 R alpha-Fc^{HCX} Chimera consists of 5-40% carbohydrate by weight.
Glycosylation	Symansis IL-10 R alpha-Fc^{HCX} Chimera has N-linked and 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 10 (IL-10) is a pleiotropic cytokine that regulates multiple immune responses through actions on T cells, B cells, macrophage/monocytes and antigen presenting cells (APC) and generally skews the immune response from TH1 to TH2. IL-10 may suppress immune responses by inhibiting expression of IL-1a, IL-1b, IL-6, IL-8, TNF-a, GM-CSF and G-CSF in activated monocytes and activated macrophages. IL-10 also suppresses IFN-g production by NK cells. The biological effects of IL-10 are mediated through binding to the IL-10 receptor (IL-10 R) complex, which belongs to the class II cytokine receptor family. The IL-10R complex is a heterodimer comprising of the IL-10 R alpha chain (IL-10 Ra) and the interleukin 10 receptor beta chain (IL-10 Rb), which is shared by cytokine receptors for IL-22, IL-28 and IL-29. The IL-10 R is expressed on the majority of leukocytes including T cells, NK cells, macrophage/monocytes, B cells, neutrophils and dendritic cells. For a recent review on IL-10 and the IL-10 receptor, please refer to Mocellin *et al.,* (2004) *Cytokine Growth Factor Rev.* **15**(1): 61-76.

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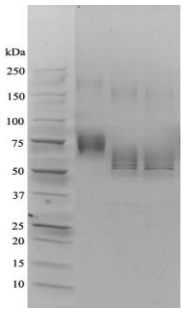
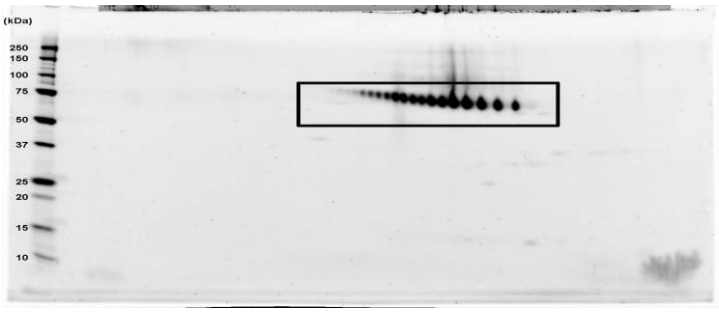
1D gel



2D gel



1D gel data

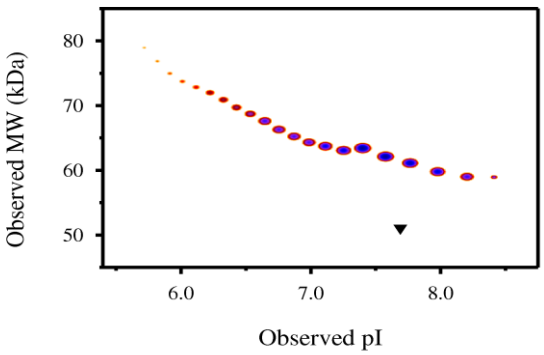
Lane 1 – MW markers; Lane 2 – IL-10 R alpha-Fc^{HCX} Chimera; Lane 3 – IL-10 R alpha-Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – IL-10 R alpha-Fc^{HCX} Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 10 ug protein loaded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

2D gel data

A sample of IL-10 R alpha-Fc^{HCX} Chimera without carrier protein was reduced, alkylated and focused on a 3-10 IPG strip then run on a 4-20% Tris-HCl 2D gel. 40 ug protein loaded. Deep Purple™ stained. Spot train indicates presence of multiple isoforms of IL-10 R alpha-Fc^{HCX} Chimera. Spots within the spot train were excised from the gel and identified as IL-10 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

HGTELPSPPSVWFEAEFFHHILHWTPIPNQSESTCYEVALLRYGIESWNSISNCSQTLSYDLTAV
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APANDTYESIFSHFREYEIAIRKVPGNFTFTHKKVKHENFSLLTSGEVGEFCVQVKPSVASRSNK
GMWSKEECISLTRQYFTGIPKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCRVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

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