SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed IL-1 RI – Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human IL-1 Receptor I (IL-1RI) (aa 1-333) 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-1RI-Fc^{HCX} Chimera migrates as a broad band between 65 and 100 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with unmodified IL-1RI-Fc that has a predicted molecular mass of 63.6 kDa.
pI	Symansis IL-1RI-Fc^{HCX} Chimera separates into a number of isoforms with a pI between 5 and 8 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IL-1RI-Fc that has a predicted pI of 7.
% Carbohydrate	Symansis purified IL-1RI-Fc^{HCX} Chimera consists of 0-36% carbohydrate by weight.
Glycosylation	Symansis IL-1RI-Fc^{HCX} Chimera has N-linked 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.
Background Information	IL-1RI is an 80kDa type I transmembrane glycoprotein expressed on T cells and fibroblasts. Structurally the extracellular domain (ECD) of IL-1R1 contains 3 Immunoglobulin like domains. Glycosylation of IL-1R1has been shown to be necessary for optimal binding of IL-1, as blocking of glycosylation sites reduces binding to IL-1. IL-1 is a proinflammatory cytokine involved in immune responses including both innate and acquired immunity and specifically stimulates T-helper cells to express cytokines associated with inflammatory responses. IL-1 also induces the expression of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) on mesenchymal cells and vascular-cell adhesion molecule-1 (VCAM-1) on endothelial cells, which promotes infiltration of inflammatory cells. Deregulation of IL-1 expression has been implicated in autoimmune disease and inhibition of IL-1 signaling alleviates joint destruction in rheumatoid arthritis. Additionally, IL-1 also increases the expression of vascular endothelial growth factor (VEGF), so may play a role in blood vessel supply in tumor progression. The functional effects of IL-1 are mediated through binding to the IL-1 receptors. There are three major receptors for IL-1 namely, IL-1RI, IL-1RII and IL-1R3, all three receptors bind to IL-1a and IL-1b while IL-1RI and IL-RII also bind to IL-1ra. For a recent review on a novel regulator of IL-1R refer to Immunol Lett. 2005 96:27-31.

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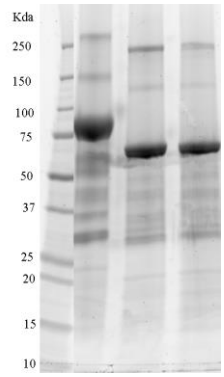
1D gel



1D gel data

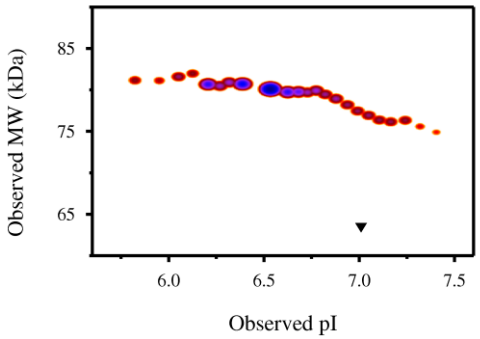
Lane 1 – MW markers; Lane 2 – IL-1RI-Fc^{HCX} Chimera; Lane 3 – IL-1RI-Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – IL-1RI-Fc^{HCX} Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 10 µg protein loaded per lane; Deep Purple™ stained.

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 available upon request.



Theoretical Sequence

LEADKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVSTEQASRIHQHKEK
LWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNAQAIFKQKLPVAGDGGLVC
PYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDRLIVMNVAEKHRGNYTCHASYTYL
GKQYPITRVIEFITLEENKPTRPVIVSPANETMEVDLGSQIQLICNVTGQLSDIAYWKWNGSVI
DEDDPVLGEDYYSVENPANKRRSTLITVLNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVT
NRSSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCRVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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