SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

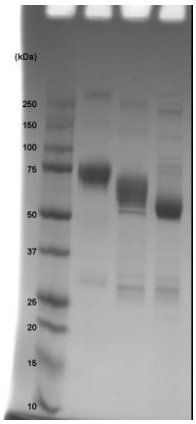
human cell expressed IFNAR2 – Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human IFNAR2 (aa 1-243) 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 IFNAR2 – Fc^{HCX} Chimera migrates as a broad band between 60 and 85 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified IFNAR2 - Fc Chimera that has a predicted monomeric molecular mass of 52 kDa.
pI	Symansis IFNAR2 – Fc^{HCX} Chimera separates into a number of isoforms with a pI between 5.0 and 6.0 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IFNAR2 – Fc Chimera that has a predicted pI of 5.75.
% Carbohydrate	Symansis purified IFNAR2 – Fc^{HCX} Chimera consists of 15-40% carbohydrate by weight.
Glycosylation	Symansis IFNAR2 – Fc^{HCX} Chimera contains N- 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.
Activity	IFNAR2 – Fc^{HCX} Chimera bound to protein A sepharose beads was able to pull down its ligand, IFNa2b.
Background Information	Type I interferon's (IFN alpha, beta, omega, tau, kappa and zeta) mediate antiviral, antitumor and immunomodulatory functions and these effects are exerted through binding to the type I interferon receptor (IFNR). This receptor is composed of 2 chains, IFNAR1 and IFNAR2. IFNAR2 is a type I membrane protein tyrosine kinase that is widely expressed in different tissues and is a member of the type II cytokine receptor family. In addition to the membrane bound form a soluble form of the IFNAR2 consisting of the extra-cellular ligand binding domain has been described with the ability to act as a Type 1 IFN agonist, by interacting with and activating cellular membrane-bound IFN receptors, or as an Type I IFN antagonist, with the potential for treatment of autoimmune diseases caused by overproduction of Type 1 IFNs. Symansis IFNAR2 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 possess enhanced affinity for its cognate ligand relative to its monomeric form. For a review on Type I Interferons please refer to Oritani *et al.,* (2001) *Cytokine Growth Factor Rev.* **12**(4): 337-48.

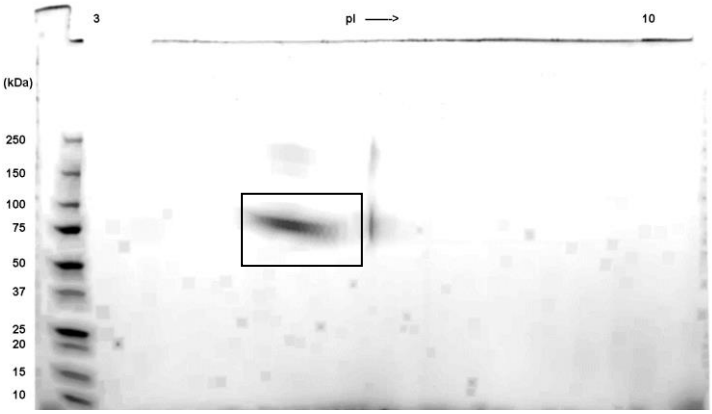
FOR RESEARCH USE ONLY

human cell expressed IFNAR2 – Fc^{HCX} Chimera

1D gel



2D Gel



1D gel data

Lane 1 – MW markers; Lane 2 – IFNAR2 – Fc^{HCX} Chimera; Lane 3 – IFNAR2 - Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycan's; Lane 4 – IFNAR2 – Fc^{HCX} Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycan's. Approximately 5 µg of protein was loaded per lane; Gel was stained using Coomassie.

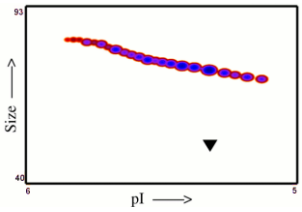
Drop in MW after treatment with PNGase F indicates presence of N-linked glycan's. A further drop in MW after treatment with the glycosidase cocktail indicates the presence of O-linked glycan's. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of IFNAR2 – 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. Approximately 40 µg of protein was load; Gel was stained using Deep Purple™. Spot train indicates presence of multiple isoforms of IFNAR2 – Fc^{HCX} Chimera. Spots within the spot train were cut from the gel and identified as IFNAR2 - Fc Chimera by protein mass fingerprinting.

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

ISYDSPDYTDESCTFKISLRNFRSILSWELKNHSIVPTHYTLLYTIMSKPEDLKVVKNCANTTR
SFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPEFEIVGFTNHINVMV
KFPSIVEEELQFDLSLVIEEQSEGIVKKHKPEIKGNMSGNFTYIIDKLIPNTNYCVSVYLEHSDE
QAVIKSPLKCTLLPPGQESESAESAKGSSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

FOR RESEARCH USE ONLY

SYMANSIS
CELL SIGNALING SCIENCE

www.symansis.com