human cell expressed IFN-gamma R2 - Fc^{hex}Chimera Data sheet

Source	DNA encoding the signal peptide and extracellular domain of human IFN-gamma R2 (aa 1-247) chain 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 IFN-gamma R2 - Fc^{hex} Chimera migrates as a broad band between 65 and 75 kDa on SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified IFN-gamma R2 - Fc Chimera that has a predicted molecular mass of 52 kDa.
pI	Symansis IFN-gamma R2 - Fc^{hex}Chimera separates into a number of glycoforms with a pI between 5.5 and 8 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IFN-gamma R2 – Fc Chimera that has a predicted pI of 7.5.
% Carbohydrate	Symansis purified IFN-gamma R2 - Fc^{hex} Chimera consists of 20-30% carbohydrate by weight.
Glycosylation	Symansis IFN-gamma R2 - Fc^{hex} Chimera has N- and possibly O-linked oligosaccharides.
Purity	>95%, as determined by SDS-PAGE and visualized by Coomassie Brilliant Blue.
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	Interferon-gamma (IFN-gamma) is a pleotropic cytokine expressed predominantly by naïve and activated CD8+ and TH1 CD4+ T cells, and natural killer (NK) cells and, as such, promotes both innate and adaptive immune responses. The activity of IFN-gamma is mediated through its receptor, the high-affinity IFN-gamma receptor complex, a class II cytokine receptor that is present on T cells, B cells, macrophages, neutrophils and NK cells as well as non-immune somatic cells such as endothelial cells and fibroblasts. The high-affinity IFN-gamma receptor complex comprises two different subunits, the alpha (IFN-gamma R1, CDw119) and beta (IFN-gamma R2) subunits. The IFN-gamma R2 subunit is a 310 amino acid (~35 kDa) type I membrane protein that contains two fibronectin type III domains and six potential N-linked glycosylation sites and is involved in signal transduction. For a review on Type I Interferons please refer to Oritani *et al.,* (2001) *Cytokine Growth Factor Rev.* **12**(4): 337-48.

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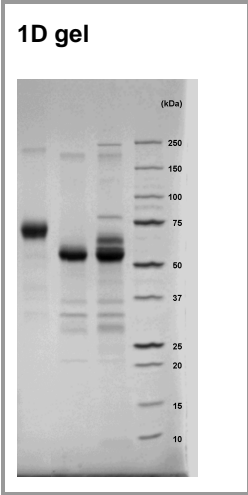
1D gel

1D gel data	Lane 1 – IFN-gamma R2 – Fc**hex** Chimera; Lane 2 – IFN-gamma R2 – Fc**hex** Chimera treated with PNGase F to remove potential N-linked glycans; Lane 3 – IFN-gamma R2 – Fc**hex** Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans; Lane 4 – MW markers. 10 µg of protein was loaded per lane; Lane 4 – MW markers; Gel was stained with Coomassie G250.

Drop in MW after treatment with PNGase F indicates the presence of N-linked glycans. Faint 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 glycoforms, which differ according to their level of post-translational modification.

Expression of these glycoforms is highly significant for cell biology, as they more closely resemble the native human proteins.
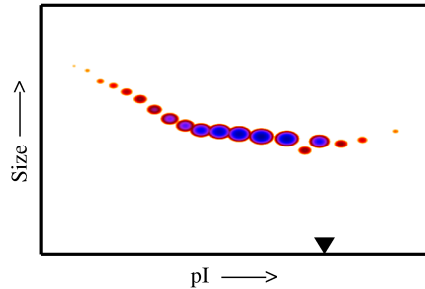
The triangle indicates theoretical pI and MW of the protein. The original 2D gel from which the densitometry scan was derived is available on request.

Theoretical Sequence

SQLPAPQHPKIRLYNAEQVLSWEPVALSNSTRPVVYRVQFKYTDSKWFTADIMSIG
VNCTQITATECDFTAASPSAGFPMDFNVTLRLRAELGALHSAWVTMPWFQHYRNV
TVGPPENIEVTPGEGSLIIRFSSPFDIADTSTAFFCYYVHYWEKGGIQQVKGPFRSN
SISLDNLKPSRVYCLQVQAQLLWNKSNIFRVGHLSNISCYETMADASTELQQGSSN
TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK