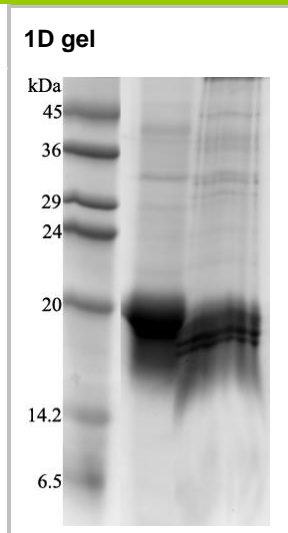


human cell expressed IFN-alpha 2b^{HGX}

Source	A DNA sequence encoding the human IFN-alpha 2b protein sequence (containing the signal peptide sequence, and the mature human IFN alpha 2b sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis IFN-alpha 2b ^{HGX} migrates as a band between 15 and 20 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified IFN-alpha 2b that has a predicted molecular mass of 19.3 kDa.
pI	Symansis IFN-alpha 2b ^{HGX} separates into a number of isoforms with a pI between 5.0 and 6.3 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IFN-alpha 2b that has a predicted pI of 5.99.
% Carbohydrate	Symansis purified IFN-alpha 2b ^{HGX} consists of 0-5% carbohydrate by weight.
Glycosylation	Symansis IFN-alpha 2b ^{HGX} has 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	The ED ₅₀ of IFN-alpha 2b ^{HGX} is typically <0.01 ng/ml as measured in a cytotoxicity assay using the growth-factor dependent TF-1 cell line.
Background Information	<p>IFN-alpha comprises a family of structurally related proteins coded for by 14 non-allelic IFN-alpha genes, one of which codes for IFN-alpha 2. Polymorphisms of the IFN-alpha 2 gene give rise to 3 isoforms, namely, IFN-alpha 2a (rarely expressed); IFN-alpha 2b (K→R at amino acid 46 of the precursor); and IFN-alpha 2c (K→R at amino acid 46, H→R at amino acid 57). IFN-alpha is produced predominantly by monocytes/macrophages, and to a lesser extent by natural killer (NK) cells, T cells, dendritic cells and plasmacytoid dendritic cells (DCs).</p> <p>The expression of IFN-alpha is induced by viral or bacterial infections and also by the components of infectious agents such as LPS, bacterial DNA and double stranded RNA. IFN-alpha treatment of DCs promotes the maturation and up-regulation of co-stimulatory molecules such as CD40, CD80, CD86 and MHC class II. This in turn facilitates antigen presentation, the priming of T cells and cytokine release to initiate immune responses. IFN-alpha anti-tumour activity includes the capacity to promote apoptosis of tumor cells and inhibit angiogenesis by inducing the down regulation of basic fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). The actions elicited by IFN-alpha result from binding to a common IFN cell surface receptor present on target cells, macrophages or DCs.</p> <p>Structurally, IFN-alpha 2 is a monomer and is O-glycosylated on Thr-129.</p> <p>For recent reviews see Moschella et al. (2003) <i>Clin Cancer Res</i> 9: 2022-31 and Bogdan (2004) <i>Immunol Rev.</i> 202: 33-48.</p>

human cell expressed IFN-alpha 2b^{HGX}



1D gel data

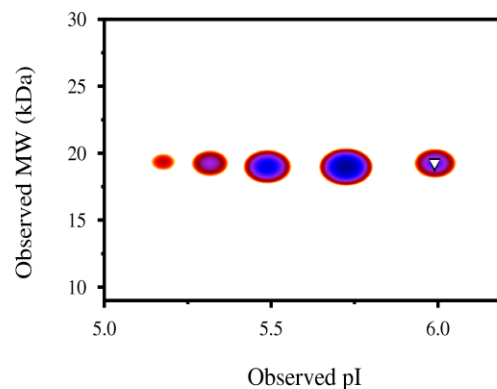
Lane 1 – MW markers; Lane 2 – IFN-alpha 2b^{HGX}; Lane 3 – IFN-alpha 2b^{HGX} treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 10 µg protein loaded per lane; Deep Purple™ stained.

Drop in MW after treatment with glycosidase cocktail indicates presence of O-linked glycans.

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

CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQI
FNLFSTKDSSAAWDETLKDFYTELYQQLNDLEACVIQGVGVTEETPLMKEDSILAVRKYFQRI
TLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE