SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed IL-12^{HCX}

Source	A DNA sequence encoding the human IL-12 protein sequence (containing the signal peptide and the mature IL-12 alpha chain sequence, and the signal peptide and the mature IL-12 beta chain sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis IL-12^{HCX} is a disulfide-linked heterodimeric glycoprotein, consisting of an alpha and beta chain. Symansis IL-12^{HCX} migrates as multiple bands between 30 and 45 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. The Symansis IL-12^{HCX} alpha chain migrates as bands between 30 and 35 kDa. This compares with the unmodified IL-12 alpha chain that has a predicted molecular mass of 22.5 kDa. The Symansis IL-12^{HCX} beta chain migrates as bands between 35 and 45 kDa. This compares with the unmodified IL-12 beta chain that has a predicted molecular mass of 34.7 kDa.
pI	Symansis IL-12^{HCX} separates into a number of isoforms with a pI between 3.5 and 9.5 in 2D PAGE due to post-translational modifications, in particular glycosylation. Symansis IL-12^{HCX} alpha chain separates into multiple isoforms with a pI between 3.5 and 9.5. This compares with the unmodified IL-12 alpha chain that has a predicted pI of 6.1. The Symansis IL-12^{HCX} beta chain separates into multiple isoforms with a pI between 5.0 and 9.0. This compares with the unmodified IL-12 beta chain that has a predicted pI of 5.4.
% Carbohydrate	Symansis purified IL-12^{HCX} alpha chain consists of 15-35% carbohydrate by weight. Symansis purified IL-12^{HCX} beta chain consists of 0-25% carbohydrate by weight.
Glycosylation	IL-12^{HCX} has N- and O-glycosylation.
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	Activity was measured by its ability to stimulate the proliferation of PHA-activated human T-lymphoblasts, and is typically in the range of 0.05-0.20 ng/ml.
Theoretical Sequence	Alpha Chain: RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVE ACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKL LMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTI DRVMSYLNAS Beta Chain: IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFG DAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCW WLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAE ESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTP HSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASV PCS

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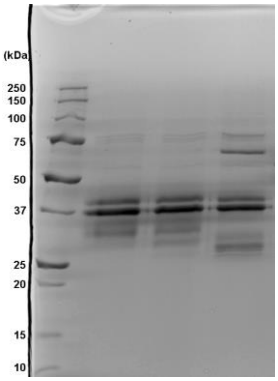
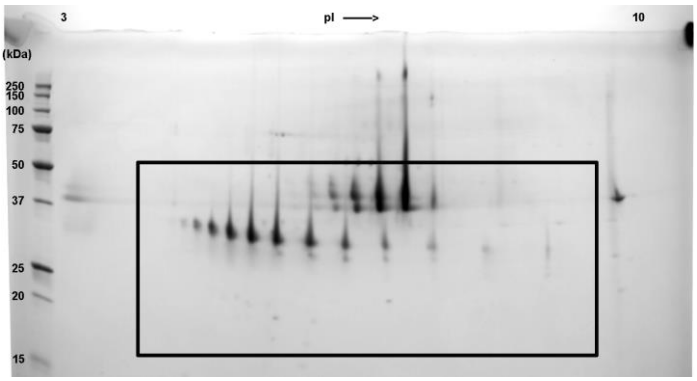
Gels

1D gel



2D gel



1D gel data

Lane 1 – MW makers; Lane 2 – IL-12^{HCX}; Lane 3 – IL-12^{HCX} treated with PNGase F to remove potential N-linked glycans, Lane 4 – IL-12^{HCX} treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 10 μg of protein was loaded per lane. Gel was stained with Deep Purple™.

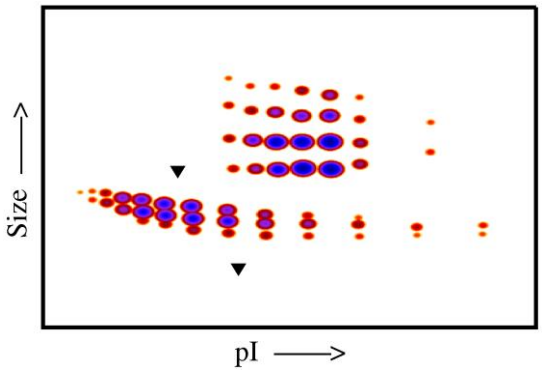
Appearance of additional band at lower MW after treatment with PNGase F indicates the presence of N-linked glycans. The subsequent drop in MW after treatment with a glycosidase cocktail indicates the presence of O-linked glycans. Additional high MW bands in lane 4 are glycosidase enzymes.

2D gel data

A sample of IL-12^{HCX} 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. 40 μg of protein was loaded. Gel was stained with Deep Purple™. Spot trains indicate presence of multiple isoforms of IL-12^{HCX}. Spots within each train were cut from the gel and identified as IL-12 by protein mass fingerprinting. Experimental details and results are available upon request.

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 triangles indicate theoretical pI and MW of the alpha and beta chains of the protein. The original 2D gel from which the densitometry scan was derived is shown above.

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human cell expressed IL-12

Background Information

Human interleukin 12 (IL-12, IL12) is a pro-inflammatory cytokine that consists of a heterodimer comprising two disulfide-linked subunits, the alpha chain (the p35 subunit, IL-12) and the beta chain (the p40 subunit, IL-12). IL-12 is synthesized as a 219 amino acid peptide including a 22 amino acid signal sequence and has 2 potential N-linked glycosylation sites and a theoretical molecular mass of approximately 22 kDa. IL-12 is synthesized as a 328 amino acid peptide including a 22 amino acid signal sequence and has two potential N-linked glycosylation sites and a theoretical molecular mass of 35 kDa.

Expression of the alpha chain and beta chain is regulated independently as the genes for the different subunits are located on different chromosomes. The majority of cell types have the ability to express the alpha chain. However, beta chain expression is restricted to dendritic cells, phagocytic cells and cells of the monocyte/macrophage lineage and B cells. It is these cells that predominantly express functional IL-12.

IL-12 production is induced by both innate and adaptive immune responses. Induction via the innate immune response involves pathogen products such as bacterial LPS and various cell wall components, CpG nucleic acids and double stranded RNA. Additionally, the process of phagocytosis of bacteria also induces IL-12.

The induction of IL-12 expression via the adaptive immune response involves the interaction of antigen presenting cells (APC) with TH cells, through CD40-CD40L interaction. Additionally, cross-linking of MHC II by TCR or CD4 induces IL-12. Importantly, at least two different signals are required for the induction of IL-12: CD40 ligation and a co-stimulatory cytokine, a bacterial product and IFN gamma, or CD40L and bacterial product.

IL-12 plays a crucial role in regulating both cell mediated and innate immunity. Specifically, it is the major cytokine responsible for inducing T helper 1 (TH1) cell, cytotoxic T-cell (CTL) and natural killer (NK) cell immune responses. Furthermore, IL-12 acts on T cells, and NK cells, stimulating proliferation and inducing the production of interferon-gamma (IFN-gamma). IL-12 also promotes the proliferation and differentiation of naive CD4+ T cells into TH1 cells that produce IFN-gamma, which in turn enhances IL-12 production in dendritic cells and phagocytes resulting in a strong positive feedback mechanism leading to a powerful cell mediated immune response.

For a review on the biology of IL-12 please refer to Gately *et. al.*, (1998) *Ann Rev Immunol* **16**: 495-521.