SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed IL-6^{HCX}

Source	A DNA sequence encoding the human Interleukin 6 protein sequence (containing the signal peptide sequence, and the mature human Interleukin 6 sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis IL-6^{HCX} migrates as a broad band between 20 and 25 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified IL-6 that has a predicted molecular mass of 21.0kDa.
pI	Symansis IL-6^{HCX} separates into a number of isoforms with a pI between 5.5 and 7.2 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IL-6 that has a predicted pI of 6.22.
% Carbohydrate	Symansis purified IL-6^{HCX} consists of 0-20% carbohydrate by weight.
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 ED50 of IL-6^{HCX} is typically 0.15 - 0.35 ng/ml as measured in a cell proliferation assay using a human growth factor-dependent TF-1 cell line.
Background Information	IL-6 is a pleotropic cytokine that regulates the development, proliferation and maturation of a number of hematopoietic cells and is essential for the maturation of B cells into immunoglobulin-secreting cells. IL-6 can also influence the growth and function of non-hematopoietic cells including the differentiation of nerve cells, metabolism of bone and induction of acute phase proteins in hepatocytes. IL-6 is predominately expressed by T cells, macrophages, fibroblasts, endothelial cells and keratinocytes. IL-6 expression can be stimulated by a number of different factors including, T cell mitogens, LPS, viruses, IL-1, TNF, IL-2, IFN-b, platelet derived growth factor (PDGF), protein kinase C, calcium ionophore A23187 and factors that increase the intracellular concentration of cAMP. IL-6 is expressed as a glycoprotein with a variable molecular mass as a result of differential glycosylation/phosphorylation patterns. At least six distinct IL-6 phosphoglycoproteins have been identified. The lower molecular weight species, 23- to 25-kDa, are O-glycosylated while the 28- to 30-kDa species are both O- and N-glycosylated. For a review of the mechanism by which the IL-6/IL-6R complex regulates the inflammatory and neoplastic state please refer to Jones SA *et al.,* (2005) *J Interferon Cytokine Res.* **25**(5): 241-53.

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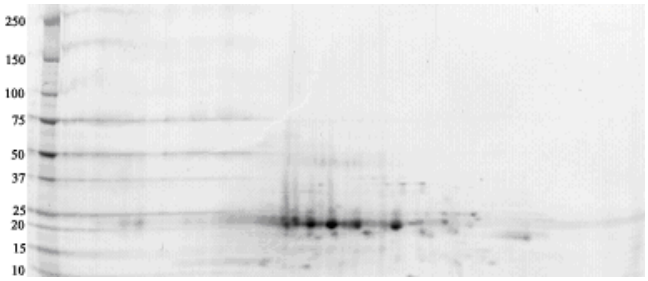
2D gel

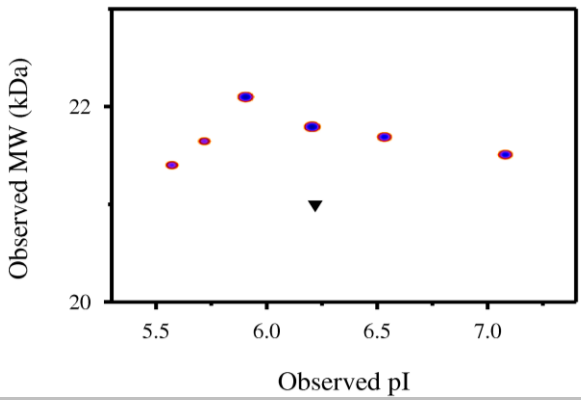


2D gel data

A sample of IL-6^{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 protein loaded per lane; Deep Purple™ stained. Spot train indicates presence of multiple isoforms of IL-6^{HCX}. Spots within the spot train were cut from the gel and identified as IL-6^{HCX} by protein mass fingerprinting. Experimental details and results are available upon request.

Densitometry

Post-translational modifications result in protein heterogeneity. The densitometry scan, derived from the 2D gel image above, 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.

Theoretical Sequence

APVPPGEDSKDVAAPHRQPLTSSERIDKQIRYILDGISALRKETCNKSNMCESSKEALAEN
NLNLPKMAEKDGCFQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQARAVQMSTKV
LIQFLQKKAKNLDAITTPDPTTNASLLTKLQAQNQWLQDMTTHLILRSFKEFLQSSLRALRQ
M