

human cell expressed IL-4<sup>HCX</sup>

Source	A DNA sequence encoding the human Interleukin 4 protein sequence (containing the signal peptide sequence, and the mature human Interleukin 4 sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis IL-4 <sup>HCX</sup> migrates as a broad band between 15 and 20 kDa on SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified IL-4 that has a predicted molecular mass of 15.0 kDa.
рІ	Symansis IL-4 <sup>HCX</sup> separates into a number of glycoforms with a pl between 9.6 and 10.0 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IL-4 that has a predicted pl of 9.26.
% Carbohydrate	Symansis purified IL-4 <sup>HCX</sup> consists of 0-25% carbohydrate by weight.
Glycosylation	Symansis IL-4 <sup>HCX</sup> has N-linked 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, with longer-term storage in aliquots at -18 to -20°C. Repeated freeze thawing is not recommended.
Activity	The ED50 of IL-4 <b>HCX</b> is typically 0.02 - 0.2 ng/ml as measured in a cell proliferation assay using the human growth factor-dependent TF-1 cell line.
Background Information	Interleukin 4 (IL-4) is a cytokine that regulates the development, proliferation and maturation of B and T-lymphocytes, as well as non-leukocyte somatic cells. IL-4 promotes antigen presentation on B cells by enhancing expression of class II MHC molecules and the co-stimulatory molecules CD80 and CD86. IL-4 can also enhance the secretion and cell surface expression of IgE and IgG1 on B-cells. The expression of the low affinity Fc receptor for IgE (CD23) on both lymphocytes and monocytes is regulated by IL-4.
	IL-4 is produced by a number of hematopoietic cell types including T-cells (predominantly $T_H2$ cells), CD3+ NK-T cells, mast cells, eosinophils, and basophils.
	Human IL-4 is synthesized as a precursor protein of 153 amino acids, which includes a hydrophobic signal sequence of 24 amino acids that is cleaved upon secretion from the cell. Biological activity of IL-4 is dependent on the formation of 3 disulfide bridges and its structure is related to that of GM-CSF, M-CSF, and growth hormone.
	For a review on IL-4 and its impact on normal and leukemic CLL B-cells please refer to Kay NE & Pittner BT (2003) <i>Leuk Lymphoma</i> . <b>44</b> (6): 897-903.



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10	) gel					
kDa						
100						
75	-				-	
50	-					
37						
25	-					
20	-					
15	-1	-	++	-	1	
10	-					

## **1D gel data** Lane 1 – MW markers; Lane 2 – IL-4**HCX**; Lane 3 – IL-4**HCX** treated with PNGase F to remove potential N-linked glycans; Lane 4 – IL-4**HCX** 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 PNGase F indicates presence of N-linked glycans. A further drop in MW after treatment with the glycosidase cocktail indicates the presence of O-linked glycans. Additional 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.



Triangle indicates theoretical pl and MW of the protein. The original 2D gel from which the densitometry scan was derived is available upon request.

## TheoreticalHKCDITLQEIIKTLNSLTEQKTLCTELTVTDIFAASKNTTEKETFCRAATVLRQFYSHHEKDTRCLSequenceGATAQQFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQSTLENFLERLKTIMREKYSKCSS

