SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed IL-3^{HCX}

Source	A DNA sequence encoding the human Interleukin 3 protein sequence (containing the signal peptide sequence, and the mature human Interleukin 3 sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis IL-3^{HCX} migrates as a broad band between 15 and 25 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified IL-3 that has a predicted molecular mass of 15.1 kDa.
pI	Symansis IL-3^{HCX} separates into a number of isoforms with a pI between 4.2 and 6.8 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IL-3 that has a predicted pI of 7.05.
% Carbohydrate	Symansis purified IL-3^{HCX} consists of 0-40% carbohydrate by weight.
Glycosylation	Symansis IL-3^{HCX} has N-linked and possibly 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	Product should be stored at 2 to 8°C. 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-3^{HCX} is typically 0.1- 0.4 ng/ml as measured in a cell proliferation assay using the human growth factor dependent TF-1 cell line.
Background Information	Interleukin-3 (IL-3) is a pleiotropic cytokine that regulates the proliferation, maturation, and survival of progenitor cells of the myeloid, erythroid, and megakaryocyte lineage. IL-3 is predominantly produced by activated T cells although expression has also been detected in monocytes and macrophages, NK-cells, mast cells, endothelial cells, and keratinocytes. IL-3 also may promote dendritic cell formation from CD34 progenitors in the presence of TNF-alpha, induce phagocytosis in stimulated macrophages, and up-regulate the expression of IL-1, IL-6, and TNF-alpha. Furthermore, IL-3 can induce the synthesis of histamines in mast cells and the expression of complement factor C3a receptors on basophils. IL-3 has also been shown to recruit eosinophils and increase platelet and neutrophil levels. IL-3 may contribute to T_H2 responses by inducing IL-10 and IL-13 in mast cells and IL-4 and IL-3 in non-B non-T cells. The potential clinical roles for IL-3 relate to its ability, either alone or in combination with G-CSF or GM-CSF, to expand haemopoietic stem cell populations. Human IL-3 is a 133 amino acid protein that contains two N-linked glycosylation sites and exists as a monomer. For a review on IL-3 in dendritic cell development and function refer to Lutz MB (2004) *Immunobiology* **209**(1-2): 79-87.

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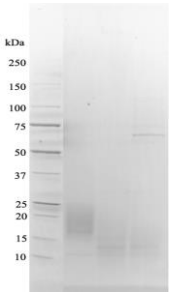
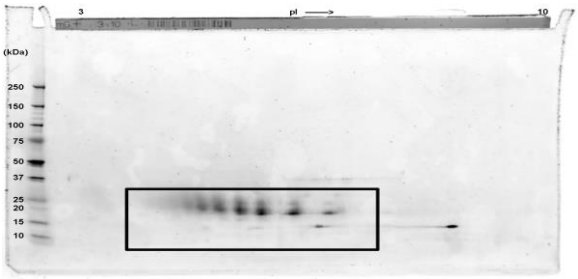
1D gel



2D gel



1D gel data

Lane 1 – MW markers; Lane 2 – IL-3^{HCX}; Lane 3 – IL-3^{HCX} treated with PNGase F to remove potential N-linked glycans; Lane 4 – IL-3^{HCX} treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 5 µg protein loaded per lane; Deep Purple™ stained. Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Faint bands in lane 3 and lane 4 are glycosidase enzymes.

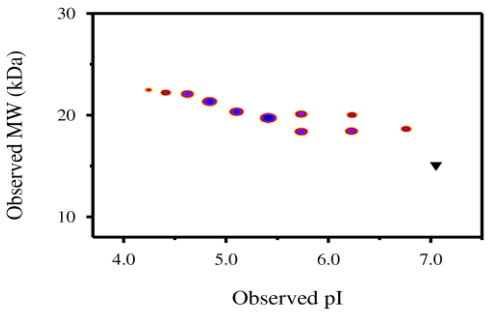
2D gel data

A sample of IL-3^{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-3^{HCX}.

Spots within the spot train were cut from the gel and identified as IL-3^{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

APMTQTTSLKTSWVNCSNMIDEIITHLKQPPLPLLDFNNLNGEDQDILMENNLRRPN
LEAFNRAVKSLQNASAIESILKNLLPCLPLATAAPTRHPIHIKDGDWNEFRRKLTFYL
KTLENAQAQQTTLSLAIF