SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed Lymphotoxin-alpha^{HCX}

Source	A DNA sequence encoding the human Lymphotoxin alpha (LT-a) protein sequence (containing the signal peptide sequence, and the mature human Lymphotoxin alpha sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis Lymphotoxin-alpha^{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 Lymphotoxin-alpha that has a predicted molecular mass of 18.7kDa.
pI	Symansis Lymphotoxin-alpha^{HCX} separates into a number of isoforms with a pI between 6 and 9 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified Lymphotoxin-alpha that has a predicted pI of 8.94.
% Carbohydrate	Symansis purified Lymphotoxin-alpha^{HCX} consists of 0-25% carbohydrate.
Glycosylation	Symansis Lymphotoxin-alpha^{HCX} contains 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 and longer-term storage of aliquots at -18 to -20°C. Repeated freeze thawing is not recommended.
Activity	The ED50 of Lymphotoxin-alpha^{HCX} is typically 0.05 - 0.07 ng/ml as measured in a cytotoxicity assay using the murine WEHI 164 cell line in the presence of actinomycin D.
Background Information	Lymphotoxin-alpha (LT-a) is a pro-inflammatory cytokine expressed in activated T cells, B cells, natural killer cells, as well as some non-hematopoietic cells. Activation signals include bacterial products, virus infection, T cell receptor activation, crosslinking of surface immunoglobulin (Ig) on B-lymphocytes, and exposure to UV light in epithelial cells. LT-a regulates a variety of biological processes including cell proliferation, apoptosis, coagulation and lipid metabolism. LT-a has been shown to be cytotoxic to a broad range of tumor cells both *in vitro* and *in vivo*. LT-a plays an important role in inflammation and protective immunity by regulating cytokine release, recruitment of effector cells to infection sites and promoting adhesion of leukocytes to endothelial cells. Human LT-a is a member of the TNF superfamily. Two forms of LT-a have been identified, a soluble trimeric complex and a heterocomplex membrane bound form that involves the association of LT-a and a related protein called lymphotoxin-beta (LT-b) The LT-a/b complex can associate as two isomers differing in the ratio of subunits present, either Lta-2/b or more commonly as LT-a/b2. LT-a is synthesized as a 205 amino acid peptide including a 34 amino acid signal sequence. LT-a has one potential N-linked glycosylation site. For further information on the role of Lymphotoxin-alpha in immunity and autoimmunity please refer to McDevitt H., et al. (2002) Arthritis Res.; 4 Suppl 3:S141-52.

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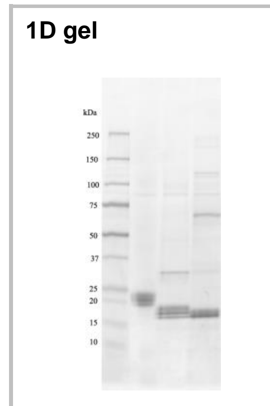


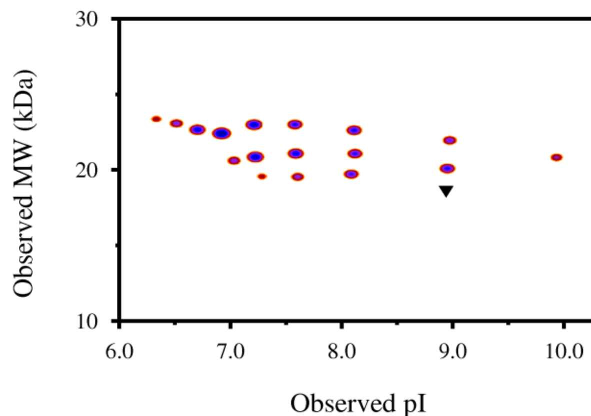
1D gel data

Lane 1 – MW markers; Lane 2 – Lymphotoxin-alpha^{HCX}; Lane 3 – Lymphotoxin-alpha^{HCX} treated with PNGase F to remove potential N-linked glycans; Lane 4 – Lymphotoxin-alpha^{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. Subsequent tightening of band after treatment with glycosidase cocktail suggests presence of O-linked glycans. Faint 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 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

LPGVGLTPSAAQTARQHPKMHLAHSNLKPAAHLIGDPSKQNSLLWRANTDRAFLQDGFS
LSNNSLLVPTSGIYFVYSQVVFSGKAYSPKATSSPLYLAHEVQLFSSQYPFHVPLLSSQK
MVYPGLQEPWLHSMYHGAAFQLTQGDQLSTHTDGIPHLVLSPSTVFFGAFAL

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