SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

Leukemia Inhibitory Factor (LIF)^{HCX} Human Cell Expressed Catalogue 3014C/D

Source	A DNA sequence encoding the human leukemia inhibitory factor (LIF) protein (comprising the signal peptide and the mature LIF) was expressed in modified human 293 cells.
Molecular Mass	Under reducing conditions Symansis LIF^{HCX}™ migrates as a broad band between 43 and 48 kDa on SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified LIF polypeptide that has a predicted monomeric molecular mass of 19.7 kDa.
pI	Symansis LIF^{HCX} ™ separates into a number of glycoforms with a pI between 5.8 and 9.9 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified LIF that has a predicted pI of 9.28.
%Carbohydrate	Symansis purified LIF^{HCX} ™ consists of 40-50% carbohydrate by weight.
Glycosylation	Symansis LIF^{HCX} ™ contains N- and O-linked oligosaccharides.
Purity	>95%, as determined by SDS-PAGE and visualized by Coomassie Brilliant Blue. Endotoxin free, molecularly tag free, and prepared under animal-free conditions.
Activity	The ED50 of Symansis LIF^{HCX} ™ is 0.5-1.5 ng/mL as measured in a cell proliferation assay using the human growth factor-dependent TF-1 cell line.
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.
Background Information	Leukemia Inhibitory Factor (LIF) is a lymphoid factor which promotes long-term maintenance of embryonic stem cells by suppressing spontaneous differentiation in rodents. LIF has a number of other activities including cholinergic neuron differentiation, control of stem cell pluripotency, bone and fat metabolism, mitogenesis of certain factor dependent cell lines and promotion of megakaryocyte production *in vivo.* Human LIF is a 19.7 kDa protein containing 181 amino acid residues. Human LIF is equally active on both human and mouse cells. Murine LIF is approximately 1000 fold less active on human cells, than hLIF. Most recently glycosylated LIF has been shown to be critical for cell functions. See " "Extensive Mannose Phosphorylation on Leukemia Inhibitory Factor (LIF) Controls Its Extracellular Levels by Multiple Mechanisms" Jarrod Barnes, Jae-Min Lim, Anne Godard, Frederic Blanchard, Lance Wells, and Richard Steet *J. Biol. Chem*. 2011 **286:** 24855-24864. Symansis is proud to offer the only human fully glycosylated form of LIF that is also molecularly tag-free, endotoxin free, and prepared under animal free conditions to help support this new line of research surrounding LIF and its function *in vivo.* For a review of LIF please see Saito *et al*. (2005) Hum Cell. **18**(3):135-41.

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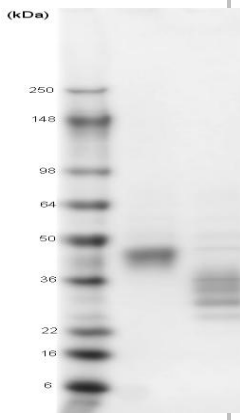
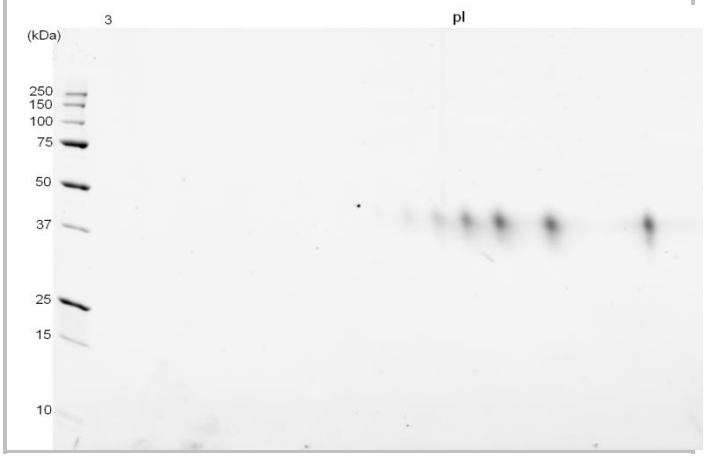
Quality Data

1D gel



2D gel



1D gel legend

Lane 1 – MW markers; Lane 2 – LIF hcx™; Lane 3 – LIF hcx™ treated with PNGase F to remove potential N-linked glycans; Lane 4 – LIF ^{HCX}™ treated with a glycosidase cocktail to remove potential N- and O-linked glycans. Approximately 5 µg of protein was loaded per lane; Gel was stained using Coomassie.

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.

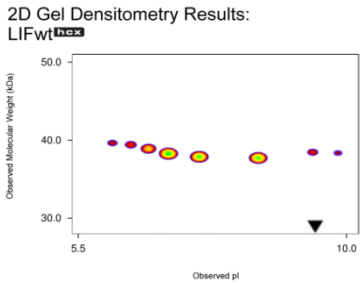
2D gel legend

A sample of LIF ^{HCX}™ without carrier protein was reduced and alkylated and focused on a 3-10 IPG strip then run on a 8-16% Tris-HCl 2D gel. Approximately 40 µg of protein was loaded; Gel was stained using Deep Purple™. Spot train indicates presence of multiple glycoforms of LIF ^{HCX}™

Densitometry

Post-translational modifications result in protein heterogeneity. The densitometry scan demonstrates that 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.

The triangle indicates theoretical pI and MW of the protein. The original 2D gel from which the densitometry scan was derived is shown above.



Theoretical Sequence	SPLPITPVNATCAIRHPCHNNLMNQIRSQLAQLNGSANALFILYYTAQGEPFPNNLDKLCGPNVTDF PPFHANGTEKAKLVELYRIVVYLGTSLGNITRDQKILNPSALSLHSKLNATADILRGLLSNVLCRLCSK YHVGHVDVTYGPDTSGKDVFQKKKLGCQLLGKYKQIIAVLAQAF