SYMANSIS
CELL SIGNALING SCIENCE

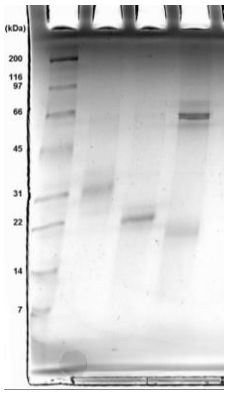
Product Data Sheet

human cell expressed SCF^{HCX}

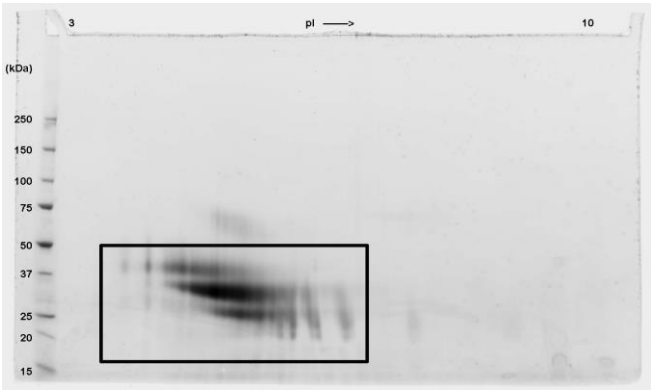
Source	A DNA sequence encoding the human stem cell factor (SCF) protein sequence (containing the signal peptide sequence, and the mature SCF sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis SCF^{HCX} migrates as a broad band between 20 and 42 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with unmodified SCF that has a predicted molecular mass of 21.0 kDa.
pI	Symansis SCF^{HCX} separates into a number of isoforms with a pI between 3.0 and 7.0 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified SCF that has a predicted pI of 5.76.
% Carbohydrate	Symansis purified SCF^{HCX} consists of 0-50% carbohydrate by weight.
Glycosylation	Symansis SCF^{HCX} contains N- and 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	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 SCF^{HCX} is typically 10-20 ng/ml as measured in a cell proliferation assay using the human growth factor-dependent M-07e cell line.
Background Information	Stem cell factor (SCF, kit ligand) is a type I membrane glycoprotein with 5 potential N-linked glycosylation sites. Soluble SCF is produced by proteolytic cleavage of the ECD. SCF is expressed primarily by fibroblasts however expression has also been detected in keratinocytes, mature granulocytes, Sertoli cells and bone marrow stromal cells. Functionally, SCF is involved in steady state maintenance of hematopoiesis and mediates the proliferation of myeloid, erythroid and lymphoid progenitors in bone marrow cultures. It has been shown to act synergistically with colony stimulating factors such as G-CSF and erythropoietin as well as interleukins. SCF is also involved in gametogenesis, melanogenesis and the membrane bound form of SCF mediates cell-cell adhesion. SCF contributes to the generation and survival of mast cells from $CD34^+$ progenitor cells and plays a role in mast cell degranulation, resulting in the release of histamine, proinflammatory cytokines and chemokines. Furthermore, SCF also induces eosinophil activation. This suggests SCF is involved in allergic disease states and elevated levels of SCF are detected in inflammatory conditions in human and animal models such as asthma. The actions of SCF are exerted by binding to the receptor tyrosine kinase c-Kit (CD117), which results in receptor dimerization. Deregulated c-Kit signalling contributes to human tumours while inhibition of c-Kit signalling reduces histamine and mast cell levels and eosinophil infiltration.

human cell expressed SCF^{HCX}

1D gel



2D gel



1D gel data

Lane 1 – MW markers; Lane 2 – SCF^{HCX}; Lane 3 – SCF^{HCX} treated with PNGase F to remove potential N-linked glycans; Lane 4 – SCF^{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 Deep Purple™.

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.

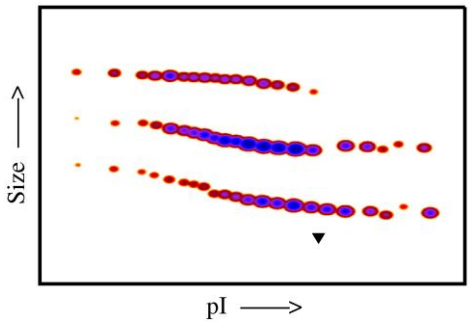
2D gel data

A sample of SCF^{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. Approximately 40 µg of protein was load; Gel was stained using Deep Purple™. Spot train indicates presence of multiple isoforms of SCF^{HCX}. Spots within the spot train were cut from the gel and identified as SCF^{HCX} by protein mass fingerprinting. Experimental details and results are available upon request.

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 shown above.



Theoretical Sequence

EGICRNRVTNNVKDVTKLVANLPKDYMITLKYVPGMDVLPSHCWISEMVVQLSDSLTDLLDK
FSNISEGLSNYSIIDKLVNIVDDLVECVKENSSKDLKKSFKSPEPRLFTPEEFFRIFNRSIDAFK
DFVVASETSDCVVSSTLSPEKDSRVSVTKPFMLPPVAASSLRNDSSSSNRKAKNPPGDSSL
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