Recombinant Human SCF sR hcx™ Product Data Sheet

human cell expressed SCF sR hcx

Source	A DNA sequence encoding the signal peptide and extracellular domain of human SCF R (aa 1-520) was expressed in modified human 293 cells.
Molecular Mass	Under reducing conditions Symansis SCF sR hcx migrates as a broad band between 65 and 100 kDa on SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified SCF sR polypeptide that has a predicted monomeric molecular mass of 55.9 kDa.
pI	Symansis SCF sR hcx separates into a number of glycoforms with a pI between 4 and 7 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified SCF sR that has a predicted pI of 6.1.
% Carbohydrate	Symansis purified SCF sR hcx consists of approximately 15-45% carbohydrate by weight.
Glycosylation	Symansis SCF sR hcx contains N- and O-linked oligosaccharides.
Purity	>95%, as determined by SDS-PAGE, visualised 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 and longer-term storage of aliquots at -18 to -20°C is recommended,. Repeated freeze thawing is not recommended.
Activity	The ED_{50} of SCF sR hcx is typically 2-4 ug/ml as by its ability to neutralise SCF mediated proliferation of the human growth dependant M-07e cell line.
Theoretical Sequence	GSSQPSVSPGEPSPPSIHPGKSDLIVRVGDEIRLLCTDPGFVKWTFEILDETNENKQNEWI TEKAEATNTGKYTCTNKHGLSNSIYVFVRDPAKLFLVDRSLYGKEDNDTLVRCPLTDPEV TNYSLKGCQGKPLPKDLRFIPDPKAGIMIKSVKRAYHRLCLHCSVDQEGKSVLSEKFILKV RPAFKAVPVVSVSKASYLLREGEEFTVTCTIKDVSSSVYSTWKRENSQTKLQEKYNSWH HGDFNYERQATLTISSARVNDSGVFMCYANNTFGSANVTTTLEVVDKGFINIFPMINTTVF VNDGENVDLIVEYEAFPKPEHQQWIYMNRTFTDKWEDYPKSENESNIRYVSELHLTRLK GTEGGTYTFLVSNSDVNAAIAFNVYVNTKPEILTYDRLVNGMLQCVAAGFPEPTIDWYFC PGTEQRCSASVLPVDVQTLNSSGPPFGKLVVQSSIDSSAFKHNGTVECKAYNDVGKTSA YFNFAFKGNNKEQIHPHT

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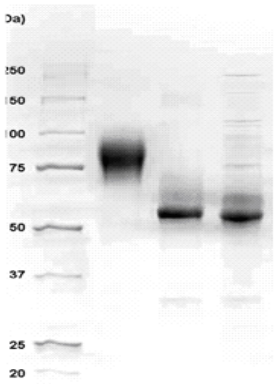
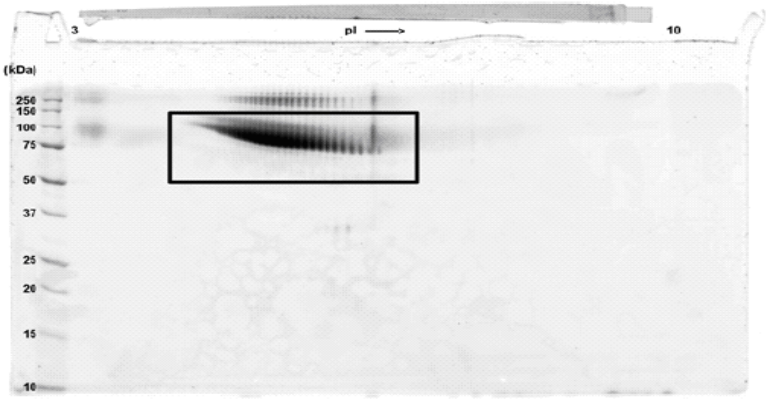
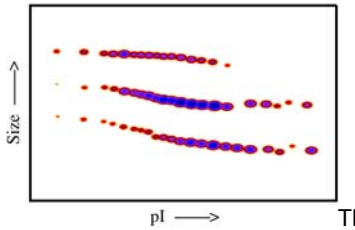
1D gel



2D gel



1D gel data	Lane 1 – MW markers; Lane 2 – SCF sR hcx; Lane 3 – SCF sR hcx treated with PNGase F to remove potential N-linked glycans; Lane 4 – SCF sR 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 Additional bands in lane 3 and lane 4 are glycosidase enzymes.
2D gel data	A sample of SCF sR 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 loaded; Gel was stained using coloidal Coomassie Brilliant Blue. Spot train indicates presence of multiple glycoforms of SCF sR hcx. Spots within the spot train were cut from the gel and identified as SCF sR hcx by mass spectrometry. Experimental details and results are available upon request.
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.

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Background Information

Stem cell factor receptor (SCF R), also known as c-kit, is a member of the type III receptor tyrosine kinase (RTK) family that includes platelet-derived growth factor (PDGF) receptors and the macrophage colony-stimulating factor 1 (CSF-1) (c-*fms*) receptor. Soluble SCF R (SCF sR) is released by human hematopoietic cells, mast cells, and endothelial cells and has been shown to bind SCF. Soluble SCF R (native or recombinant) can be a potent SCF antagonist.

SCF R is essential for the development of normal hematopoietic cells and plays an important role in the survival, proliferation, and differentiation of mast cells, melanocytes, and germ cells. It is expressed by hematopoietic cells in the embryonic liver throughout development, and by more committed progenitors, such as myeloid, erythroid, megakaryocytic, natural killer, and dendritic progenitor cells.

A variety of human diseases are associated with the inappropriate expression or activation of SCF R. The overexpression of SCF R has previously been documented in myeloid leukemia, neuroblastoma, breast tumor, colon tumors, gynecological tumors, testicular germ cell tumors and small cell lung carcinoma (SCLC). Loss-of-function mutations in SCF R can result in diseases such as autosomal-dominant piebaldism, leading to deafness, megacolon, and abnormalities in pigmentation of skin and hair.

For a recent review please refer to Roskoski, R. Jr,. (2005) *Biochem. Biophys. Rec. Commun.* **338**(3):1307-1315.

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