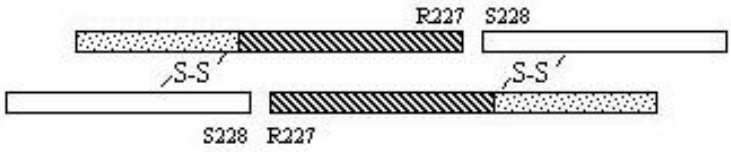
SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed VEGF-C^{HCX} secreted form - homodimeric precursor

Source	A DNA sequence encoding the human stem cell factor (VEGF-C) protein sequence (containing the signal peptide sequence, pro-peptides and the mature VEGF-C sequence) was expressed in modified human 293 cells.
Molecular Mass	The Symansis VEGF-C^{HCX} secreted antiparallel homodimer migrates between 45 and 55 kDa due to post-translational modifications, in particular glycosylation. This compares with the unmodified homodimer that has a predicted molecular mass of 43.9 kDa. Individual chains of Symansis VEGF-C^{HCX} migrate as a band between 27 and 35 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified VEGF-C chains that have a predicted molecular mass of 21.6 and 22.2 kDa.
pI	Symansis VEGF-C^{HCX} secreted antiparallel homodimer separates into a number of isoforms with a pI between 5.6 and 8.4 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified VEGF-C homodimer that has a predicted pI of 7.8
% Carbohydrate	Symansis purified VEGF-C^{HCX} consists of 15-40% from 1D gel of individual chains carbohydrate by weight
Glycosylation	Symansis VEGF-C^{HCX} contains N- and probably 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.
Molecule schematic	

Schematic diagram illustrating the disulfide linked antiparallel homodimeric VEGF-C molecule. N-terminal propeptide (mottled); C-terminal propeptide (open); VEGF homology domain (striped); disulfide bonds marked as –S-S-; Arginine 227 marked as R227; Serine 228 marked as S228. Modified from Joukov et al., (1997) EMBO J 16:3898-3911.

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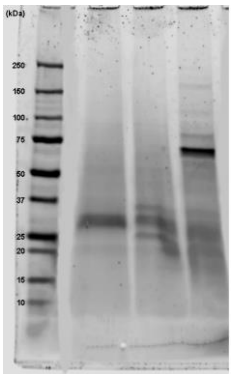
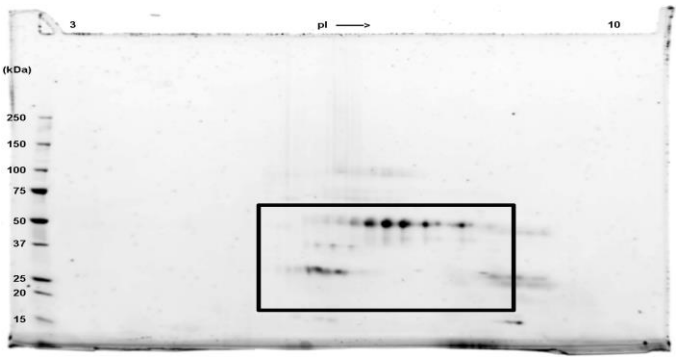
1D gel



2D gel



1D gel data

Lane 1 – MW markers; Lane 2 – VEGF-C^{HCX}; Lane 3 – VEGF-C^{HCX} treated with PNGase F to remove potential N-linked glycans; Lane 4 – VEGF-C^{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 possible further drop in MW after treatment with the glycosidase cocktail indicates the possible presence of O-linked glycans. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

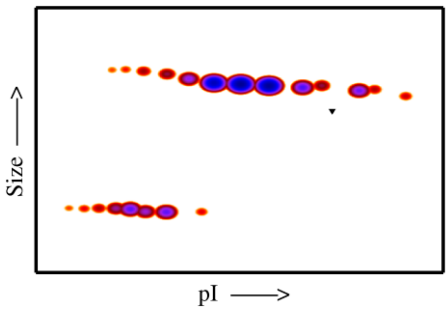
2D gel data

A sample of VEGF-C^{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 VEGF-C^{HCX}. Spots within the spot train were cut from the gel and identified as VEGF-C^{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.



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Background Information

Vascular endothelial growth factor C (VEGF-C), also known as Flt4 ligand (Flt4-L), vascular endothelial growth factor-related protein (VRP) and VEGF-2, is a member of the cysteine-knot growth factor superfamily and exhibits homology with VEGF-121 (32%) and PDGF (27%). However VEGF-C also possesses a 180 amino acid C-terminal cysteine-rich domain absent in other VEGFs.

The major secreted form of VEGF-C is a disulfide linked antiparallel homodimeric protein containing 5 potential N-linked glycosylation sites and has a theoretical molecular weight of 44 kDa. Synthesis and secretion of VEGF-C from the cell involves a series of cleavage steps to produce the mature protein. The first step is antiparallel precursor homodimerization followed by proteolytic cleavage between Arg-227 and Ser-228 dividing the VEGF-C precursor into 2 nearly equal parts. This is followed by slow proteolytic cleavage to remove the N-terminal propeptide, with most of the VEGF-C secreted by different cell types being found as the disulfide linked antiparallel homodimeric precursor, rather than as the mature protein.

The actions of VEGF-C are exerted through VEGF Receptor-2 (VEGFR-2) and VEGF Receptor-3 (VEGFR-3) however only the fully cleaved VEGF-C can activate VEGF2 receptor. Thus it is hypothesized that the complex proteolytic processing pathway of VEGF-C is used to prevent unnecessary angiogenic effects, elicited via VEGFR-2 which is present in many types of endothelia, and allows VEGF-C to signal preferentially via VEGFR-3, which has restricted expression. Please see Joukov et al., (1997) EMBO J 16:3898-3911 for further information.

VEGF-C is predominantly involved in angiogenesis, vasculogenesis, lymphangiogenesis as well as an inhibitor of dendritic cell maturation. VEGF-C also possesses both mitogenic and chemotactic activity w.r.t. endothelial cells and monocytes. VEGF-C is over-expressed in a number of carcinomas such as breast and colorectal carcinoma and has been shown to enhance metastasis to regional lymph nodes and to the lungs.

For a review on the biology of VEGFs, including VEGF-C, please refer to Ferrara N et al., (2003) Nat Med. 9(6): 669-76 and Carmeliet (2005) Nature 438: 932-936.

Theoretical Sequence

FESGLDLSDAEPDAGEATAYASKDLEEQLRSVSSVDELMTVLYPEYWKMYKCQLRKGGW
QHNREQANLNSRTEETIKFAAAHYNTEILKSIDNEWRKTQCMPREVCIDVGKEFGVATNTFF
KPPCVSVYRCGGCCNSEGLQCMNTSTSYLSKTLFEITVPLSQGPKPVTISFANHTSCRCMS
KLDVYRQVHSIIRR

Disulfide bonded to:

SLPATLPQCQAANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELD
EETCQCVCRAGLRPASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRT
CPRNQPLNPGKCACECTESPQKCLLKGKKFHHQTCSCYRRPCTNRQKACEPGFSYSEEV
CRCVPSYWKRPQMS

Note: N-terminal sequences confirmed by Edman sequencing.