SYMANSIS
CELL SIGNALING SCIENCE

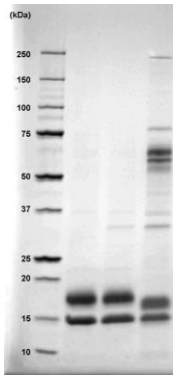
Product Data Sheet

human cell expressed VEGF-121^{HCX}

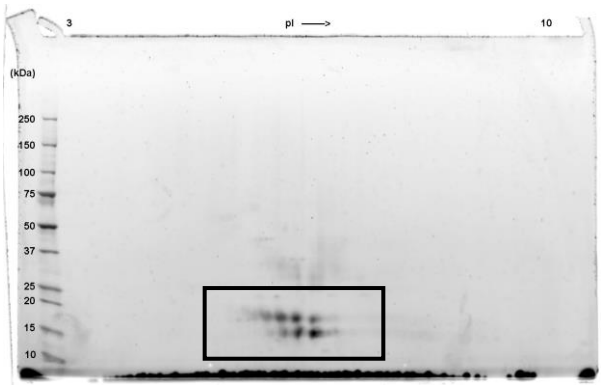
Source	A DNA sequence encoding the human splice isoform VEGF-121 protein sequence (containing the signal peptide sequence, and the mature human VEGF-121 sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis VEGF-121^{HCX} migrates as a two bands between 14.5 and 20 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with unmodified VEGF-121 that has a predicted molecular mass of 14 kDa.
pI	Symansis VEGF-121^{HCX} separates into a number of isoforms with a pI between 5.5 and 7.0 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified VEGF-121 that has a predicted pI of 6.1.
% Carbohydrate	Symansis purified VEGF-121^{HCX} consists of 0-30% carbohydrate by weight.
Glycosylation	Symansis VEGF-121^{HCX} contains 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 ED_{50} of VEGF-121^{HCX} is typically 0.5-2.5 ng/ml as measured in a cell proliferation assay using human umbilical vein endothelial (HUVEC) cells.
Background Information	Vascular endothelial growth factor (VEGF, VEGF-A, VPF) is a covalently linked homodimeric glycoprotein (45-48 kDa) that is a member of the cysteine-knot growth factor superfamily. VEGF-121 is a splice variant isoforms of VEGF-A. VEGF-121 is a freely soluble mitogen that does not bind heparin since it lacks two exons (6 and 7), the presence of which confers heparin binding ability. As a consequence, VEGF121 is able to diffuse more freely because it does not bind to heparan-sulfate proteoglycans, yet it is fully active as an inducer of angiogenesis and as a blood vessel permeabilizing agent. Studies have revealed that VEGF-121 is essential for normal embryonic development and plays a major role in the physiological and pathological events of angiogenesis in adults. VEGF-121 has also been shown to play an important role during endochondral bone formation in hypertrophic cartilage remodelling. Structurally, VEGF-121 exists as disulfide linked homodimer and contains 2 potential N-linked glycosylation sites. For a review on the biology of VEGF please refer to Ferrara N *et al.,* (2003) *Nat Med.* **9**(6): 669-76.

human cell expressed VEGF-121^{HCX}

1D gel



2D gel



1D gel data

Lane 1 – MW markers; Lane 2 – VEGF-121^{HCX}; Lane 3 – VEGF-121^{HCX} treated with PNGase F to remove potential N-linked glycans; Lane 4 – VEGF-121^{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 G250.

The Absence of a drop in MW after treatment with PNGase F indicates that there are no N-linked glycans attached to the protein. A subsequent 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

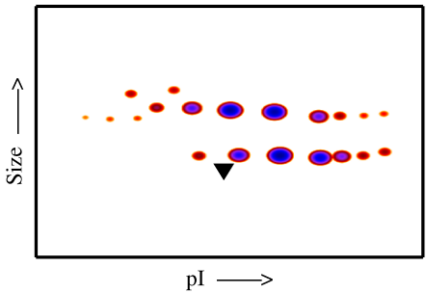
A sample of VEGF-121^{HCX} without carrier protein was reduced and alkylated and focused on a 3-10 IPG strip then run on a 4-20% Tris-HCl 2D gel. Approximately 40 µg of protein was load; Gel was stained using Deep Purple™.

Multiple spot trains indicate the presence of multiple isoforms of VEGF-121^{HCX}. Spots within both spot trains were cut from the gel and identified as VEGF-121^{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.



Theoretical Sequence

APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCC
NDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDRARQEKCDKPRR

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