Product Data Sheet

Human Cell Expressed NGF R (209 aa) –Fc HCX Chimera Catalogue # 9015

Source	A DNA sequence encoding the signal peptide and extracellular domain of human NGF receptor (aa 1-237) was fused to the Fc region of human IgG1 (aa 93-330). The chimeric protein was expressed in modified human 293 cells.
Molecular Mass	Symansis NGF R (209 aa) – Fc HCX Chimera migrates as a broad band between 65 and 90 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified NGFR-Fc Chimera that has a predicted mass of 49.2kDa.
pI	Symansis NGF R (209 aa) - Fc HCX Chimera separates into a number of isoforms with a pI between 4.2 and 5.3 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified NGF R-Fc Chimera that has a predicted pI of 4.89.
% Carbohydrate	Symansis purified NGF R (209 aa) - Fc HCX Chimera consists of 25-45% carbohydrate by weight.
Glycosylation	Symansis NGF R (209 aa) - Fc HCX Chimera has N-linked 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.
Theoretical Sequence	KEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPC TECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDKQN TVCEECPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIPGRWITRSTP PEGSDSTAPSTQEPEAPPEQDLIASTVAGVVTTVMGIPKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCRVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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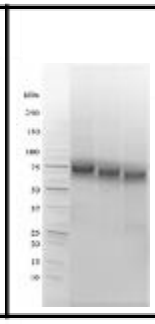
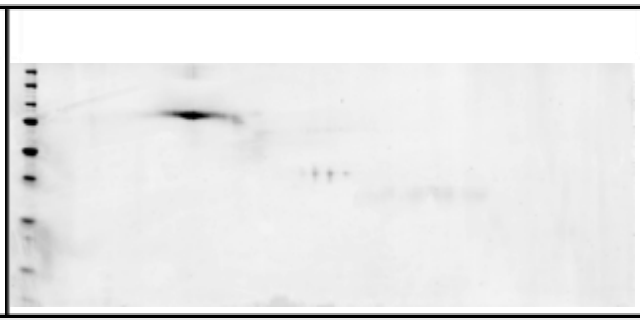
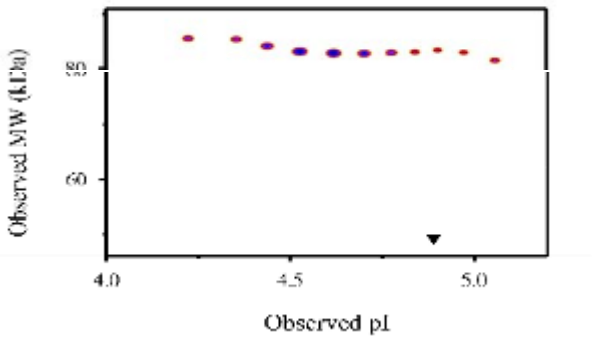
	1D gel	2D gel
1D gel data		
	Lane 1 – MW markers; Lane 2 – NGF R (209 aa) - Fc HCX Chimera; Lane 3 – NGF R (209 aa) - Fc HCX Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – NGF R (209 aa) - Fc HCX Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 10 µg protein loaded per lane; Deep Purple™ stained.	
2D gel data	Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Subsequent drop in MW after treatment with glycosidase cocktail indicates the presence of O-linked glycans. Faint bands in lane 3 and lane 4 are glycosidase enzymes.	
	A sample of NGF R (209 aa) - Fc HCX Chimera 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. 40 µg protein loaded per lane; Deep Purple™ stained.	
	Spot train indicates presence of multiple isoforms of NGF R (209 aa) - Fc HCX Chimera. Spots within the spot train were cut from the gel and identified as NGF R (209 aa) - Fc HCX Chimera by protein mass fingerprinting. Experimental details and results are available upon request.	

Densitometry

Post-translational modifications result in protein heterogeneity. The densitometry scan, derived from the 2D gel image above, 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.

Triangle indicates theoretical pI and MW of the protein.



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Background Information

Nerve growth factor receptor (NGF R; NGFR) is a low affinity NGF receptor. NGF R binds with equal affinity all neurotrophins including NGF beta, brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) and neurotrophin-5 (NT4/5). The association of NGF R with the other NGF receptors such as TrkA, B and C results in higher affinity ligand binding. Ligand binding to the NGF R can promote either survival or apoptosis of neurons.

The effects of neurotrophins exerted through NGF R include conditions such as pain, depression, obesity, nerve regeneration disorders, learning and memory. Additionally, NGF R may play a role in neuronal death that occurs in disorders of the CNS such as Alzheimer's disease.NGF R is a type I membrane protein that is synthesized as a 427 amino acid glycoprotein comprised of a 28 amino acid signal peptide, a 222 amino acid extracellular domain that includes four TNFR-Cys repeats (aa31-aa188), a Ser/Thr rich stalk (aa197-aa248), a 22 amino acid transmembrane region, and a 155 amino acid cytoplasmic domain. NGF R is N-glycosylated and phosphorylated on serine residues, and mass spectroscopic analysis of the NGF R stalk identified 7 sites of O-linked glycosylation that may affect the affinity of neurotrophin binding (see Chapman et al., 1996 J. Neurochem. 66, 1707-1716).

Symansis Life Sciences' NGF R (209 aa) does not contain the aforementioned stalk. In contrast to TrkA, B and C, which contain intracellular tyrosine kinase domains, NGF R lacks intracellular enzymatic activity. However NGF R does contain a type II death domain for binding TNF receptor associated factors (TRAFs) that function in mediating the effects of NGF R signaling.

For a review of NGF R and Alzheimer's disease please refer to Salehi A, et al. (2004) J Neural Transm. 111(3): 323-45.