SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed TrkB-Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human TrkB (aa 1-428) 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 TrkB-Fc^{HCX} Chimera migrates as a broad band between 90 and 115 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified TrkB-Fc Chimera that has a predicted molecular mass of 71.0kDa.
pI	Symansis TrkB-Fc^{HCX} Chimera separates into a number of isoforms with a pI between 4.9 and 6.7 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified TrkB-Fc Chimera that has a predicted pI of 5.79.
% Carbohydrate	Symansis purified TrkB-Fc^{HCX} Chimera consists of 20-40% carbohydrate by weight.
Glycosylation	Symansis TrkB-Fc^{HCX} Chimera has N-linked and may have 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	Symansis TrkB-Fc^{HCX} Chimera bound to protein A sepharose beads is able to pull down its ligand, BDNF.
Background Information	The tropomyosin-related kinase (Trk) family consists of TrkA, TrkB and TrkC. These receptors each bind to and are activated by one or more of the neurotrophins, which include neural growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophic factors 3 and 4/5 (NT-3, NT-4/5). TrkB is a high affinity receptor for BDNF and NT-4/5, and also binds NT-3 with lower affinity. TrkB is widely expressed, primarily in nervous tissue. The biological effects of TrkB activation are crucial in neuronal differentiation during embryonic development, specifically in the formation of the sympathetic nervous system. Additionally, the TrkB/BDNF complex facilitates the survival of post-mitotic neurons, axon growth and guidance as well as synaptic plasticity. TrkB also contributes to learning and spatial memory formation and the attenuation of injury-induced neuronal cell death. In addition to regulating CNS physiology TrkB and BDNF are involved in the formation of follicular growth and oocyte survival in the mammalian ovary.TrkB is a heavily glycosylated type I transmembrane protein of 791 amino acids, and the TrkB-Fc Chimera contains 13 potential N-linked glycosylation sites. For a review of the role of TrkB in neuronal signal transduction please refer to Huang EJ & Reichardt LF (2003) *Annu Rev Biochem*. 72: 609-42.

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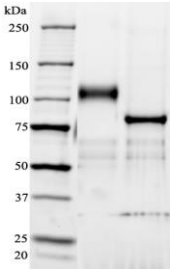
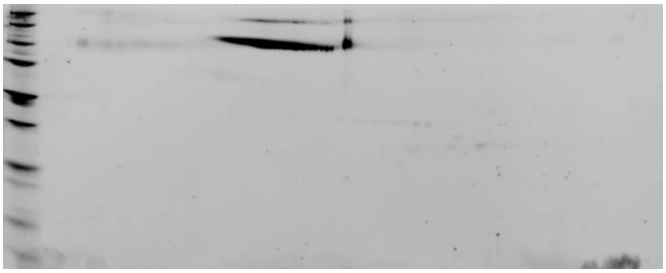
1D gel data

1D gel



2D gel



Lane 1 – MW markers; Lane 2 – TrkB-Fc^{HCX} Chimera; Lane 3 – TrkB-Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans. 10 µg protein loaded per lane; Deep Purple™ stained. Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Band in lane 3 at 35 kDa is PNGase F.

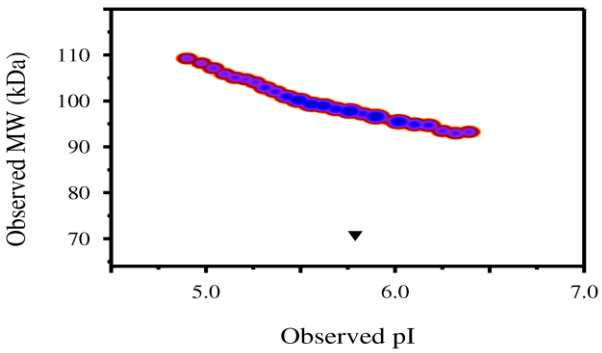
2D gel data

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

The triangle indicates theoretical pI and MW of the protein.



Theoretical Sequence

CPTSCKCSASRIWCSDPSPGIVAFPRLEPNSVDPENITEIFIANQKRLEIINEDDVEAYVGLRNL
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LQEAKSSPDTQDLYCLNESSKNIPLANLQIPNCGLPSANLAAPNLTVEEGKSITLSCSVAGDPV
PNMYWDVGNLVSKHMNETSHTQGSLRITNISSDDSGKQISCVAENLVGEDQDSVNLTVHFAP
TITFLESPTSDHHWCIPFTVKGNPKPALQWFYNGAILNESKYICTKIHVTNHTEYHGCLQLDNP
THMNNGDYTLIAKNEYGKDEKQISAHFMGWPGIDDGANPNYPDVIYEDYGTAANDIGDTTNR
SNEIPSTDVTDKTGRIPKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK