SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed TrkC – Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human TrkC (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 TrkC – Fc^{HCX} Chimera migrates as a broad band between 85 and 150 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified TrkC – Fc Chimera that has a predicted molecular mass of 71.9 kDa.
pI	Symansis TrkC – Fc^{HCX} Chimera separates into a number of isoforms with a pI between 4 and 7 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified TrkC – Fc Chimera that has a predicted pI of 5.8.
% Carbohydrate	Symansis purified TrkC – Fc^{HCX} Chimera consists of 15-55% carbohydrate by weight.
Glycosylation	TrkC – Fc^{HCX} Chimera is N-glycosylated and is probably O-glycosylated.
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.
Background Information	TrkC, also known as neurotrophic tyrosine kinase receptor type 3 (NTRK3), is a member of the tropomyosin-related kinase (trk or Trk) receptor family, consisting of TrkA, TrkB, and TrkC. Trk receptors bind neurotrophins, including neural growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophic factors 3 and 4/5 (NT-3, NT4/5). TrkC is located on the plasma membrane of neural cells such as the hippocampus, the cerebral cortex, and the granular cell layer of the cerebellum, and it specifically binds NT-3 as a ligand. TrkC is a heavily glycosylated molecule with 13 potential N-linked glycosylation sites within the extracellular domain (ECD). TrkC has been implicated in the development of leukemias via gene fusion with the TEL gene and treatment with TrkC agonist antibodies delays disease progression of neuromuscular degeneration in susceptible mice. NT-3 has also been implicated in chronic pain and therefore, the sequestering of NT-3 by a soluble TrkC receptor could be used as a treatment against a number of pathological conditions involving chronic pain, including inflammatory, neuropathic, and cancer pain. For a recent review, please refer to Huang EJ, Reichardt LF (2003) *Annu. Rev. Biochem.* **72**: 609-642.

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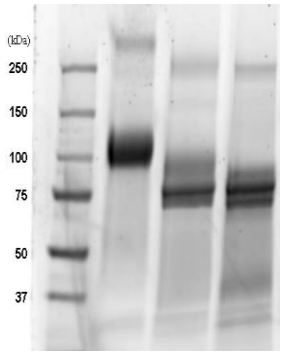
Gels

1D gel



1D gel data

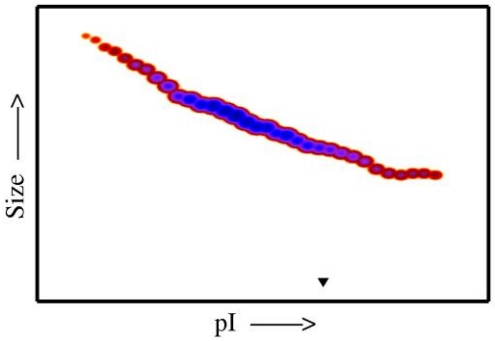
Lane 1: MW makers; Lane 2: TrkC – Fc^{HCX} Chimera; Lane 3: TrkC – Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4: TrkC – Fc^{HCX} Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 5 μg of protein was loaded per lane. Gel was stained with Deep Purple™.

Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Subsequent tightening of bands after treatment with glycosidase cocktail suggests the presence of O-linked glycans.

Densitometry

Post-translational modifications result in protein heterogeneity. The densitometry scan demonstrates that 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 triangles indicate theoretical pI and MW of the alpha and beta chains of the protein. The original 2D gel from which the densitometry scan was derived is available.



Theoretical Sequence

CPANCVCSKTEINCRRPDDGNLFPLLEGQDSGNSNGNANINITDISRNITSIHIENWRSLHTLN
AVDMELYTGLQKLTIKNSGLRSIQPRAFAKNPHLRYINLSSNRLTTLSWQLFQTLSLRELQLE
QNFFNCSCDIRWMQLWQEQGEAKLNSQNLYCINADGSQLPLFRMNISQCDLPEISVSHVNL
TVREGDNAVITCNGSGSPLPDVDWIVTGLQSINTHQTNLNWTNVHAINLTLVNVTSEDNGFTL
TCIAENVVGMSNASVALTVYYPPRVVSLEEPELRLEHCIEFVVRGNPPPTLHWLHNGQPLRE
SKIIHVEYYQEGEISEGCLLFNKPTHYNNGNYTLIAKNPLGTANQTINGHFLKEPFPESTDNFIL
FDEVSPTPPITVTHKPEEDGSSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK