SYMANSIS
CELL SIGNALING SCIENCE

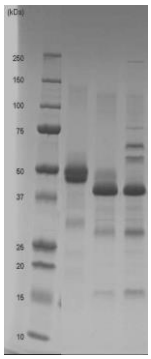
Product Data Sheet

human cell expressed TRAIL R1 – Fc^{HCX} Chimera

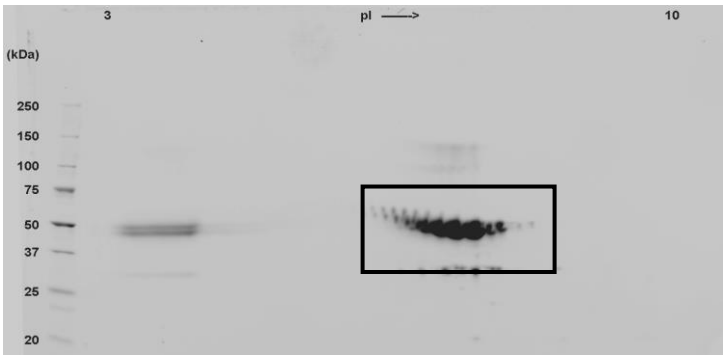
Source	A DNA sequence encoding the signal peptide and extracellular domain of human TRAIL R1 (aa 1-239) was fused to the Fc region of human IgG1 (aa 90-330). The chimeric protein was expressed in modified human 293 cells.
Molecular Mass	Under reducing conditions Symansis TRAIL R1 – Fc^{HCX} Chimera migrates as a broad band between 45 and 60 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified TRAIL R1 - Fc Chimera that has a predicted monomeric molecular mass of 50.1 kDa.
pI	Symansis TRAIL R1 – Fc^{HCX} Chimera separates into a number of isoforms with an observed pI between 6.0 and 9.0 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified TRAIL R1 - Fc Chimera that has a predicted pI of 8.8.
% Carbohydrate	Symansis purified TRAIL R1 – Fc^{HCX} Chimera consists of 10 - 35% carbohydrate by weight.
Glycosylation	Symansis TRAIL R1 – Fc^{HCX} Chimera contains N- 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.
Activity	The ED_{50} of TRAIL R1-Fc^{HCX} Chimera is typically 8-14 ng/ml as measured by its ability to neutralize TRAIL mediated cytotoxicity using human leukemic Jurkat cells.
Background Information	TRAIL R1, also known as death receptor 4, TNFRSF10A and TNF-related apoptosis-inducing ligand receptor 1, is a type 1 transmembrane protein that contains a death domain in its cytoplasmic domain. TRAIL R1 is a receptor for the cytotoxic ligand TRAIL/TNFSF10. Upon binding to TRAIL, the receptor elicits an apotopic death response. TRAIL R1 is widely expressed with high levels found in the spleen, small intestine, thymus and peripheral blood leukocytes. Symansis TRAIL R1 is produced as an ECD-Fc fusion protein with the aim of enhancing its activity. ECD-Fc fusion proteins have an advantage over soluble receptors because many receptors are only functional in dimeric form. Fusion to the Fc domain of IgG1 induces dimerization due to the ability of the Fc domain to form disulfide bonds. The resulting dimeric receptor ECD-Fc mimics the activated form of the receptor and possess enhanced affinity for its cognate ligand relative to its monomeric form. Symansis TRAIL R1- Fc Chimera neutralizes this ability of TRAIL to induce an apotopic death response. For a recent review please see Zauli and Secchiero (2006) Cytokine & Growth Factor Reviews 17:245-257.

human cell expressed TRAIL R1 – Fc^{HCX} Chimera

1D gel



2D Gel



1D gel data

Lane 1 – MW markers; Lane 2 – TRAIL R1 – Fc^{HCX} Chimera; Lane 3 – TRAIL R1 – Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – TRAIL R1 – Fc^{HCX} Chimera 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.

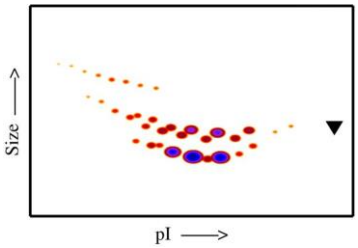
Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. A tightening of the band after treatment with the glycosidase cocktail indicates the presence of O-linked glycans. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of TRAIL R1 – 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. Approximately 40 µg of protein was load; Gel was stained using Deep Purple™. The spot train indicates the presence of multiple isoforms of TRAIL R1 - Fc^{HCX} Chimera. Spots within the spot train were cut from the gel and identified as TRAIL R1 – 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 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.

Theoretical Sequence

ASGTEAAAATPSKVWGSSAGRIEPRGGGRGALPTSMGQHGPSARARAGRAPGP
RPAREASPRLRVHKTFKFVVVGVLLQVVPSSAATIKLHDQSIGTQQWEHSPLGELC
PPGSHRSEHPGACNRCTEGVGYTNASNNLFACLPCTACKSDEEERSPCTTTRNTA
CQCKPGTFRNDNSAEMCRKCSRGCPRGMVKVKDCTPWSDIECVHKESGNGHNG
SSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

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