SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed TRAIL R2 – Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human TRAIL R2 (aa 1-182) 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 R2 – Fc^{HCX} Chimera migrates as a broad band between 40 and 50 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified TRAIL R2 - Fc Chimera that has a predicted monomeric molecular mass of 41.5 kDa.
pI	Symansis TRAIL R2 – Fc^{HCX} Chimera separates into a number of isoforms with a pI between 6.0 and 8.0 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified TRAIL R2 - Fc Chimera that has a predicted pI of 6.5.
% Carbohydrate	Symansis purified TRAIL R2 – Fc^{HCX} Chimera consists of 0–25% carbohydrate by weight.
Glycosylation	Symansis TRAIL R2 – 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 R2 – Fc^{HCX} Chimera is typically 38-40 ng/ml as measured by its ability to neutralize TRAIL mediated cytotoxicity using the human leukemic Jurkat cells.
Background Information	TRAIL R2, also known as death receptor 5, TNFRSF10B and TNF-related apoptosis-inducing ligand receptor 2, is a type 1 transmembrane protein that contains a death domain in its cytoplasmic domain. TRAIL R2 is a receptor for the cytotoxic ligand TRAIL/TNFSF10. Upon binding to TRAIL, the receptor elicits an apotopic death response. TRAIL R2 is widely expressed in adult and fetal tissues. In particular, there are high levels of expression can be found in the heart, liver, pancreas, spleen, thymus, prostate, ovary, uterus, placenta, testis, esophagus, stomach, intestines and peripheral blood lymphocytes, TRAIL R2 is not detectable in the brain. Symansis TRAIL R2 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 possesses enhanced affinity for its cognate ligand relative to its monomeric form. Symansis TRAIL R2- 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.

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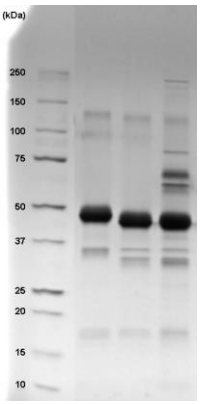
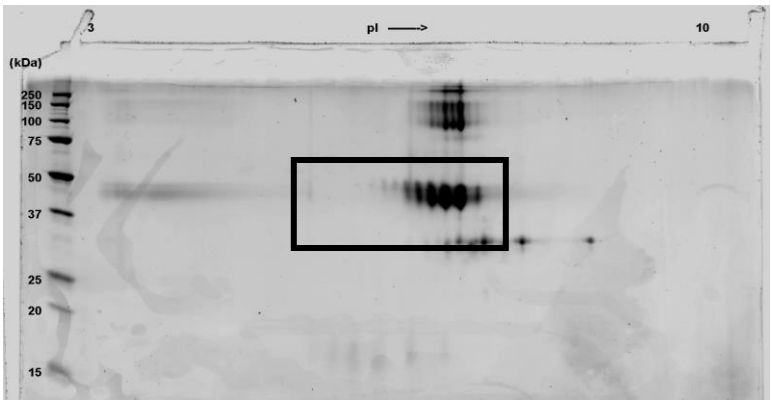
1D gel



2D Gel



1D gel data

Lane 1 – MW markers; Lane 2 – TRAIL R2 – Fc^{HCX} Chimera; Lane 3 – TRAIL R2 – Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – TRAIL R2 – 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.

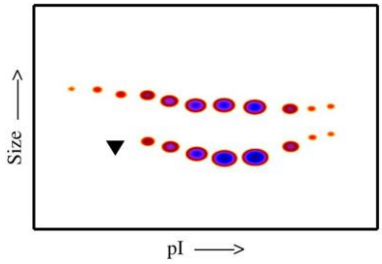
Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. A further drop in MW after treatment with the glycosidase cocktail indicates the presence of O-linked glycans. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of TRAIL R2 – 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 R2 - Fc^{HCX} Chimera. Spots within the spot train were cut from the gel and identified as TRAIL R2 – 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

ITQQDLAPQQRAAPQQKRSSPSEGLCPPGHHISEDGRDCISCKYGQDYSTHWNDLLFCL
RCTRCDSGEVELSPCTTTRNTVCQCEEGTFREEDSPEMCRKCRTGCPRGMVKVGDCT
PWSDIECVHKEGSSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

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