SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

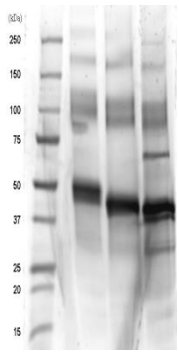
human cell expressed Cripto-1 – Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human Cripto-1 (aa 1-169) 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 Cripto-1 – Fc^{HCX} Chimera migrates as a broad band between 45 and 55 kDa on SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified Cripto-1 - Fc Chimera that has a predicted monomeric molecular mass of 42.8 kDa.
pI	Symansis Cripto-1 - Fc^{HCX} Chimera separates into a number of glycoforms with an observed pI between 6.5 and 10 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified Cripto-1 - Fc Chimera that has a predicted pI of 8.2.
% Carbohydrate	Symansis purified Cripto-1 – Fc^{HCX} Chimera consists of 5-30% carbohydrate by weight.
Glycosylation	Symansis Cripto-1 – Fc^{HCX} Chimera contains N-linked and O-linked oligosaccharides.
Purity	>95%, as determined by SDS-PAGE and visualized by Coomassie Briliant Blue.
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, with longer-term storage in aliquots at -18 to -20°C. Repeated freeze thawing is not recommended.
Activity	200 ng/ml Symansis Cripto-1 - Fc^{HCX} Chimera induces ERK1 and ERK2 phosphorylation in human umbilical vein endothelial (HUVEC) cells
Background Information	Cripto-1 (CR-1), also known as teratocarcinoma-derived growth factor-1 (TDGF-1), is a small glycoprotein that contains a single divergent EGF-like motif as well as a novel cysteine-rich domain termed the Cripto motif. It is the founding member of the EGF-CFC gene family, which is conserved among vertebrates, with homologs in chick, frogs, and zebrafish. Molecular genetic studies in fish and mice have revealed that EGF-CFC proteins play essential roles in early embryonic development in specification of the anterior-posterior and left-right body axes, as well as in formation of the primary germ layers during gastrulation. Cripto-1 is also an oncogenic growth factor involving tumorigenesis and cancer cell proliferation and survival. It is also believed that Cripto-1 may be involved in stem cell maintenance. For a recent review please see Strizzi *et al.* (2005) Oncogene 24:5731-5741

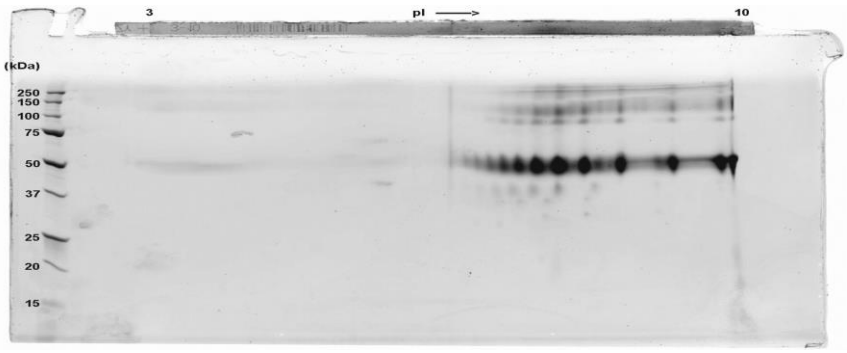
FOR RESEARCH USE ONLY

human cell expressed Cripto-1 – Fc^{HCX} Chimera

1D gel



2D Gel



1D gel data

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

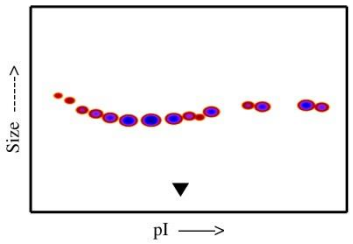
Drop in MW after treatment with PNGase F indicates the presence of N-linked glycans. A further drop in MW after treatment with the glycosidase cocktail indicates the presence of O-linked glycans. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of Cripto-1 – 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 loaded; Gel was stained using Deep Purple™. The spot train indicates the presence of multiple glycoforms of Cripto-1 – Fc^{HCX} Chimera Spots within the spot train were cut from the gel and identified as Cripto-1 – 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 glycoforms, which differ according to their level of post-translational modification. Expression of these glycoforms 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

LGHQEFARPSRGYLAFRDDSIWPQEEPAIRPRSSQRVPPMGIQHSKELNRTCCL
NGGTCMLGSFCACPPSFYGRNCEHDVRKENCGSVPHDTWLPKKCSLCKCWHG
QLRCFPQAFLPGCDGLVMDEHLVASRTPELPPSGSSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

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