SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

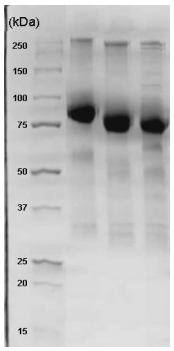
human cell expressed CRC-beta – Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human CRC-β (aa 1-443) 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 CRC-β – Fc^{HCX} Chimera migrates as a broad band between 75 and 100 kDa on SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified CRC-β - Fc Chimera that has a predicted monomeric molecular mass of 76 kDa.
pI	Symansis CRC-β – Fc^{HCX} Chimera separates into a number of glycoforms with a pI between 5.5 and 7.5 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified CRC-β - Fc Chimera that has a predicted pI of 6.1.
% Carbohydrate	Symansis purified CRC-β – Fc^{HCX} Chimera consists of 0-25% carbohydrate by weight.
Glycosylation	Symansis CRC-β – Fc^{HCX} Chimera contains N- and O-linked oligosaccharides.
Purity	>95%, as determined by SDS-PAGE and visualized by Coomassie Brilliant 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.
Background Information	CRC-β (cytokine receptor common beta chain, CRC-beta, beta c) is the beta common receptor chain of GM-CSF receptor, IL-5 receptor and IL-3 receptor. For example, human GM-CSF receptor is composed of an alpha and a beta subunit. The beta subunit does not bind to GM-CSF by itself, however when assocated with the alpha subunit (GM-CSF Rα) it forms a high affinity receptor. CRC-β has 3 potential N-glycosylation sites located in the extracellular domain. N-glycosylation of the beta subunit is essential for GM-CSF binding and signalling [Niu *et al.* (2000) Blood 95:3357-3362]. For a recent review please see Scott CL and Begley CG (1999) Int J Biochem Cell Biol 31:1011-1015.

FOR RESEARCH USE ONLY

human cell expressed CRC-beta – Fc Chimera

1D gel



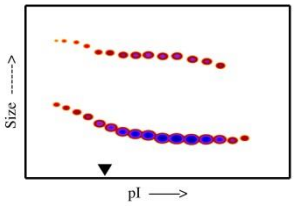
1D gel data

Lane 1 – MW markers; Lane 2 – CRC-β – Fc^{HCX} Chimera; Lane 3 – CRC-beta – Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – CRC-beta - 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 Deep Purple™.

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.

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 available on request.

Theoretical Sequence

WERSLAGAEETIPLQTLRCYNDYTSHITCRWADTQDAQRLVNVTLIRRVNEDLL
EPVSCDLSDDMPWSACPHPRCVPRRCVIPCQSFVVTDVDYFSFQPDRPLGTR
LTVTLTQHVQPPEPRDLQISTDQDHFLLTWSVALGSPQSHWLSPGDLEFEVVY
KRLQDSWEDAAILLSNTSQATLGPEHLMPSSTYVARVRTRLAPGSRLSGRPSK
WSPEVCWDSQPGDEAQPQNLECFFDGAAVLSCSWEVRKEVASSVSFGLFYK
PSPDAGEEECSPVLREGLGSLHTRHHCQIPVPDPATHGQYIVSVQPRRAEKHI
KSSVNIQMAPPSLNVTKDGDSYSLRWETMKMRYEHIDHTFEIQYRKDTATWKD
SKTETLQNAHSMALPALEPSTRYWARVRVRTSRTGYNGIWSEWSEARSWDT
ESVLPMWGSSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FOR RESEARCH USE ONLY