SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed GM-CSF R alpha – Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human GM-CSF Rα (aa 1-320) 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 GM-CSF Rα – Fc^{HCX} Chimera migrates as a broad band between 75 and 100 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified GM-CSF Rα - Fc Chimera that has a predicted monomeric molecular mass of 61.7 kDa.
pI	Symansis GM-CSF Rα – Fc^{HCX} Chimera separates into a number of isoforms with a pI between 6.0 and 9.5 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified GM-CSF Rα - Fc Chimera that has a predicted pI of 8.7.
% Carbohydrate	Symansis purified GM-CSF Rα – Fc^{HCX} Chimera consists of 15-40% carbohydrate by weight.
Glycosylation	Symansis GM-CSF Rα – Fc^{HCX} Chimera contains N-linked oligosaccharides and may contain 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	Symansis GM-CSF Rα – Fc^{HCX} Chimera bound to protein A sepharose beads was able to pull down its ligand, GM-CSF.
Backg ound Information	Human GM-CSF receptor is composed of an alpha and a beta subunit. The alpha subunit (GM-CSF Rα) binds to GM-CSF, and when assoicated with the beta subunit (CRC beta, Common Receptor Chain beta, GM-CSF Rβ) it forms a high affinity receptor. GM-CSF Rα has 11 potential N-glycosylation sites all located in the extracellular domain. N-glycosylation of the alpha subunit is essential for GM-CSF binding and signalling [Ding *et al.* (1995) J Biol Chem 270:24580-24584]. Symansis GM-CSF Rα 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. For a recent review please see Baj *et al.* (2000) Eur J Gynaecol Oncol 21:305-308.

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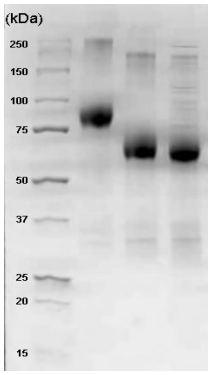
1D gel



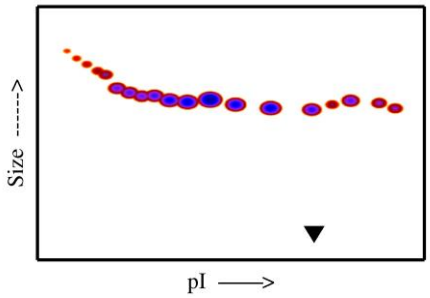
1D gel data

Lane 1 – MW markers; Lane 2 – GM-CSF Rα – Fc^{HCX} Chimera; Lane 3 – GM-CSF Rα – Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – GM-CSF Rα – 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 tightening of the band after treatment with the glycosidase cocktail indicates O-linked glycans may be present. 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 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 available on request.

Theoretical Sequence

EKSDLRTVAPASSLNVRFDSRTMNLSWDCQENTTFSKCFLTDKKNRVVEPRLSN
NECSCTFREICLHEGVTFEVHVNTSQRGFQQKLLYPNSGREGTAAQNFSCFIYNA
DLMNCTWARGPTAPRDVQYFLYIRNSKRRREIRCPYYIQDSGTHVGCHLDNLSG
LTSRNYFLVNGTSREIGIQFFDSLLDTKKIERFNPPSNVTVRCNTTHCLVRWKQPR
TYQKLSYLDFQYQLDVHRKNTQPGTENLLINVSGDLENRYNFPSSEPRAKHSVKI
RAADVRILNWSSWSEAIEFGSDDGGSSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK