SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed G-CSF R - Fc^{hex} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human G-CSF R (aa 1-621) 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 G-CSF R - Fc^{hex} Chimera migrates as a broad band between 100 and 130 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with unmodified TRAIL R1 - Fc Chimera that has a predicted monomeric molecular mass of 93.9 kDa.
pI	The unmodified G-CSF R - Fc Chimera has a predicted pI of 6.6.
% Carbohydrate	Symansis purified G-CSF R - Fc^{hex} Chimera consists of 5-30% carbohydrate by weight.
Glycosylation	Symansis G-CSF R - Fc^{hex} 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℃. Following reconstitution short-term storage at 4℃ is recommended, and longer-term stor age of aliquots at -18 to -20℃. Repeated freeze thawing is not recommended.
Activity	The ED_{50} of G-CSF R – Fc^{hex} Chimera is typically 2-3 ng/ml as measured by its ability to neutralize G-CSF mediated proliferation of the murine myeloblastic m-NFS-60 cell line.
Background Information	G-CSF R, also known as granulocyte colony-stimulating factor receptor and CD114 antigen, is a member of the cytokine receptor superfamily. Members of this family, which also includes the receptors for IL-2 to IL-7, GM-CSF, EPO, TPO and GH, are characterised by the presence of four conserved cysteine residues in their extracellular domains. The G CSF R extracellular domain has a composite structure containing an Ig-like domain, a cytokine receptor homologous region and fibronectin type III domains. The human G-CSF R mediates the biological activity of G-CSF which is unique in its ability to not only stimulate the proliferation but also potently induces the terminal maturation of myeloid progenitor cells to neutrophilic granulocytes. Symansis G-CSF R 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 comprehensive review please see Avalos BR (1996) Blood 88(3): 761-777

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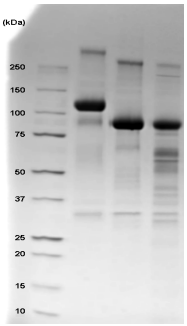
1D gel



1D gel data	Lane 1 – MW markers; Lane 2 – G-CSF R - Fc^{hex} Chimera; Lane 3 – G-CSF R - Fc^{hex} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – G-CSF R - Fc^{hex} 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 that O-linked glycans may be present. Additional bands in lane 3 and lane 4 are glycosidase enzymes.
Theoretical Sequence	ECGHISVSAPIVHLGDPITASCIIKQNCSHLDPEPQILWRLGAELQPGGRQQRLSDGTQES IITLPHLNHTQAFLSCCLNWGNSLQILDQVELRAGYPPAIPHNLSCLMNLTTSSLICQWEP GPETHLPTSFTLKSFKSRGNCQTQGDSILDCVPKDGQSHCCIPRKHLLLYQNMGIWVQA ENALGTSMSPQLCLDPMDVVKLEPPMLRTMDPSPEAAPPQAGCLQLCWEPWQPGLHIN QKCELRHKPQRGEASWALVGPLPLEALQYELCGLLPATAYTLQIRCIRWPLPGHWSDWS PSLELRTTERAPTVRLDTWWRQRQLDPRTVQLFWKPVPLEEDSGRIQGYVVSWRPSGQ AGAILPLCNTTELSCTFHLPSEAQEVALVAYNSAGTSRPTPVVFSESRGPALTRLHAMAR DPHSLWVGWEPPNPWPQGYVIEWGLGPPSASNSNKTWRMEQNGRATGFLLKENIRPF QLYEIIVTPLYQDTMGPSQHVYAYSQEMAPSHAPELHLKHIGKTWAQLEWVPEPPELGK SPLTHYTIFWTNAQNQSFSAILNASSRGFVLHGLEPASLYHIHLMAASQAGATNSTVLTLM TLTPRSSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK