SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

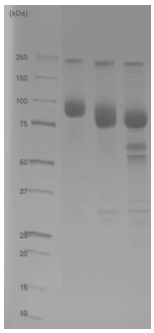
human cell expressed EGF R – Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide and extracellular domain of human EGF R (aa 1-525) 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 EGF R – Fc^{HCX} Chimera migrates as a broad band between 85 and 110 kDa on SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with unmodified EGF R – Fc Chimera that has a predicted monomeric molecular mass of 83 kDa.
pI	Symansis EGF R – Fc^{HCX} Chimera separates into a number of glycoforms with a pI between 6.0 and 8.5 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified EGF R - Fc Chimera that has a predicted pI of 7.8.
% Carbohydrate	Symansis purified EGF R – Fc^{HCX} Chimera consists of 5-30% carbohydrate by weight.
Glycosylation	Symansis EGF R – 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.
Activity	The ED_{50} of EGF R – Fc^{HCX} Chimera is typically 60 -100 ng/ml as measured by its ability to neutralize EGF mediated proliferation of murine NIH3T3 fribroblasts.
Background Information	EGF R, also known as Epidermal growth factor receptor, is a member of the ErbB family receptors. EGF R is present in most, if not all, normal human epithelial cells. EGF R main function is as a receptor for EGF, but it also acts as a receptor for other members of the EGF family such as TGF-alpha, amphiregulin, and heparin-binding EGF-like growth factor. When EGF attaches to human EGF R it activates the enzyme tyrosine kinase, triggering reactions that cause the cells to grow and multiply. EGF R is a 479 amino acid glycoprotein that consists of an extracellular receptor domain, a transmembrane region, and an intracellular domain with tyrosine kinase function. For a recent review please see Herbst RS (2004) Int. J. Radiation Oncology Biol. Phys., 59(2): 21-26

FOR RESEARCH USE ONLY

human cell expressed EGF R – Fc^{HCX} Chimera

1D gel



1D gel data

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

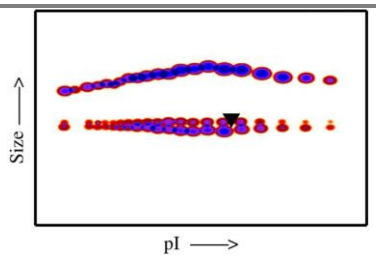
Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. A further drop in MW after treatment with the glycosidase cocktail indicates the presence of O-linked glycans. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of EGF R – 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™. Spot train indicates presence of multiple glycoforms of EGF R - Fc^{HCX} Chimera. The spots within the spot train were cut from the gel and identified as EGF R – 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 available on request.



Theoretical Sequence

LEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEVVLGNLEITYVQRNYDLSFLKTIQEVA
GYVLIALNTVERIPLENLQIIRGNMYYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRF
SNNPALCNVESIQWRDIVSSDFLSNMSMDFQNHLGSCQKCDPSCPNGSCWGAGEENCQK
LTKIICAQQCSGRCRGKSPSDCCHNQCAAGCTGPRESDCLVCRKFRDEATCKDTCPPLMLY
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EGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDI
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DVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPR
DCVSRSSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

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