SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

Human cell expressed Activin RIA – Fc Chimera^{HCX}

Source	A DNA sequence encoding the signal peptide and extracellular domain of human Activin RIA (aa 1-123) 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 Activin RIA – Fc Chimera^{HCX} migrates as a broad band between 40 and 48 kDa on SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified Activin RIA - Fc Chimera that has a predicted monomeric molecular mass of 38.7 kDa.
pI	Symansis Activin RIA - Fc Chimera^{HCX} separates into a number of glycoforms with an observed pI between 5.5 and 8.5 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified Activin RIA - Fc Chimera that has a predicted pI of 6.8.
% Carbohydrate	Purified Activin RIA - Fc Chimera^{HCX} consists of 3-20% carbohydrate by weight.
Glycosylation	Symansis Activin RIA - Fc Chimera^{HCX} contains N-linked oligosaccharides and may contain 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	Activin RIA (also known as ACVR1 or ALK-2) is an activin type I receptor. The activin type I receptors transducer signals for a variety of members of the transforming growth factor (TGF) beta superfamily of ligands. This family of cytokine and hormones include activin, anti-müllerian hormone (AMH), bone morphogenetic proteins (BMPs) and nodal. Despite the large amount of processes that these ligands regulate, they all operate through essentially the same pathway: A ligand binds to a type II receptor, which recruits and trans-phosphorylates a type I receptor. The type I receptor recruits a receptor regulated SMAD (R-SMAD) which it phosphorylates. The R-SMAD then translocates to the nucleus where it functions as a transcription factor. Activin RIA is expressed in normal parenchymal cells, endothelial cells, fibrobasts and tumor-derived epithelial cells. Defects in the receptor are a cause of fibrodysplasia ossificans progressive (FOP). For a recent review please see Chen *et al.* (2006) Exp Biol Med 231:534-544.

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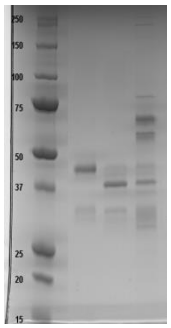
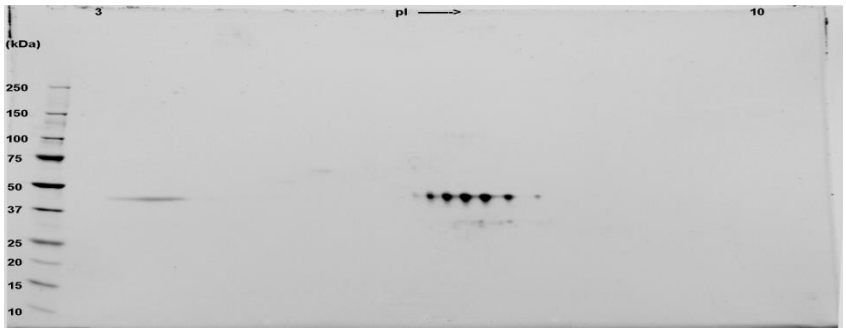
1D gel



2D Gel



1D gel data

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

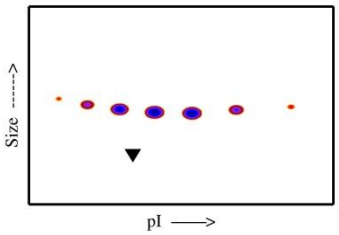
Drop in MW after treatment with PNGase F indicates the presence of N-linked glycans. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of Activin RIA - 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 iglycoforms of Activin RIA - Fc^{HCX} Chimera. Spots within the spot train were cut from the gel and identified as Activin RIA – 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 iglycoforms, 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

MEDEKPKVNPKLYMCVCEGLSCGNEDHCEGQQCFSSLSINDGFHVYQKGCFQVYEQGK
MTCKTPPSPGQAVECCQGDWCNRNITAQLPTKGKSFPGTQNFHLEGSSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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