SYMANSIS
CELL SIGNALING SCIENCE

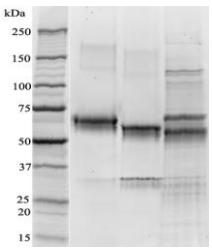
Product Data Sheet

human cell expressed Ox40-Fc^{HCX} Chimera

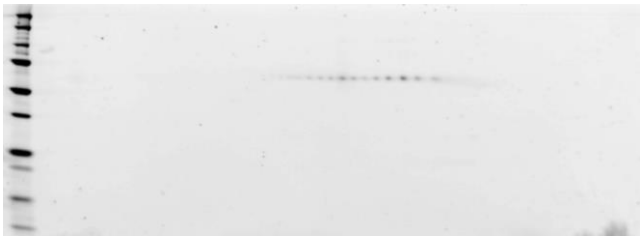
Source	A DNA sequence encoding the signal peptide and extracellular domain of human Ox40 (aa 1-207) was fused to the Fc region of human IgG1 (93-330). The chimeric protein was expressed in modified human 293 cells.
Molecular Mass	Symansis Ox40-Fc^{HCX} Chimera igrates as a broad band between 55 and 65 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified Ox40-Fc Chimera that has a predicted molecular mass of 46.4kDa.
pI	Symansis Ox40-Fc^{HCX} Chimera separates into a number of isoforms with a pI between 5.5 and 7.9 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified Ox40-Fc Chimera that has a predicted pI of 8.32.
% Carbohydrate	Symansis purified Ox40-Fc^{HCX} Chimera consists of 15-30% carbohydrate by weight.
Glycosylation	Symansis Ox40-Fc^{HCX} Chimera has N-linked and 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.
Background Information	Ox40 is a member of the tumor necrosis factor receptor (TNFR) family. It is a type I transmembrane protein expressed primarily on the surface of activated CD4+ T-cells. It is also expressed at low levels on the surface of activated CD8+ T cells, B cells, dendritic cells and eosinophils. Ox40 is a co-stimulatory molecule involved in T-cell activation and proliferation, the induction of cytokine production by effector T-cells, generation of memory T-cells, and arresting peripheral T-cell tolerance *in vivo*. Expression of Ox40 is induced following the initiation of a CD28 signal. It has been reported that the interaction of Ox40 with its ligand plays a role in the expansion of T-cell numbers at the height of the immune response as well as the generation of memory T-cells. Human Ox40 comprises a 186 amino acid extracellular domain containing four TNFR-Cys repeats, followed by a 21 amino acid transmembrane domain and a 42 amino acid cytoplasmic domain. Ox40 activity is mediated by NF-kappaB signaling via interaction of the intracellular domain of Ox40 with TRAF proteins. Deregulated levels of Ox40 or an imbalance between Ox40 and Ox40L may result in T-cell mediated diseases especially allergic, inflammatory and autoimmune diseases. Additionally, Ox40 may also be important in the enhancement of anti-tumor responses. For a review of the potential of Ox40 as a clinical target please refer to Weinberg AD (2002) *Trends Immunol*. 23(2): 102-9.

human cell expressed Ox40-Fc^{HCX} Chimera

1D gel



2D gel



1D gel data

Lane 1 – MW markers; Lane 2 – Ox40-Fc^{HCX} Chimera; Lane 3 – Ox40-Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – Ox40-Fc^{HCX} Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 10 µg protein loaded per lane; Deep Purple™ stained.

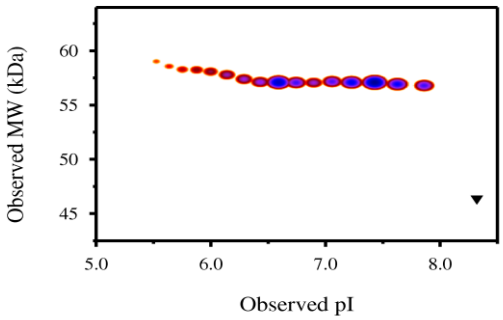
Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Slight drop in MW after treatment with glycosidase cocktail suggests presence of O-linked glycans. Additional bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of Ox40-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. 40 µg protein loaded per lane; Deep Purple™ stained. Spot train indicates presence of multiple isoforms of Ox40-Fc^{HCX} Chimera. Spots within the spot train were cut from the gel and identified as Ox40-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, derived from the 2D gel image above, 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. Triangle indicates theoretical pI and MW of the protein.



Theoretical Sequence

LHCVGDTYPSNDRCCHECRPGNGMVSRCSRSQNTVCRPCGPGFYNDVVSSKPCKP
CTWCNLRSGSERKQLCTATQDTVCRCRAGTQPLDSYKPGVDCAPCPPGHFSPGDNQ
ACKPWTNCTLAGKHTLQPASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWP
RTSQGPSTRPVGIPKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCRVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

SYMANSIS
CELL SIGNALING SCIENCE

FOR RESEARCH USE ONLY
www.symansis.com