SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

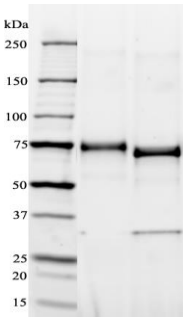
human cell expressed CD209L-Fc^{HCX} Chimera

Source	A DNA sequence encoding the signal peptide of IL-10 receptor alpha chain was fused to the extracellular domain of human CD209L (aa 1-328), which was in turn fused to the Fc region of human IgG1 (aa 93-330). The chimeric protein was expressed in modified human 293 cells.
Molecular Mass	Symansis CD209L-Fc^{HCX} Chimera migrates as a broad band between 70 and 85 kDa on SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified CD-209L-Fc that has a predicted molecular mass of 65.6 kDa.
pI	Symansis CD209L-Fc^{HCX} Chimera separates into a number of glycoforms on 2D PAGE due to post-translational modifications, in particular glycosylation. The pI range is between 5.4 and 6.5. This compares with the unmodified CD209L-Fc Chimera that has a predicted pI of 5.70.
% Carbohydrate	Symansis's purified CD209L-Fc^{HCX} Chimera consists of 5-25% carbohydrate by weight.
Glycosylation	CD209L-Fc^{HCX} Chimera has N-linked and may have 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, with longer-term storage in aliquots at -18 to -20°C. Repeated freeze thawing is not recommended.
Background Information	CD209L / DC-SIGNR, a member of the 'SIGN' family of C-type lectin receptors, is a type II membrane protein that is expressed on liver sinusoidal endothelial cells (LSEC), specialized capillary vessels that are involved in antigen presentation and hepatic immune surveillance. CD209L is also expressed by endothelial cells associated with lymph nodes and in the human lung on type II alveolar cells and endothelial cells. Structurally CD209L shares 77% amino acid sequence homology with DC-SIGN, a dendritic cell expressed homolog, and comprises the following domains; the cytoplasmic domain, a transmembrane domain, and an extracellular domain comprising a neck region with seven repeats of a 23 amino acid sequence and a carbohydrate recognition domain (CRD). There are 4 potential glycosylation sites within the extracellular domain of CD209L. CD209L binds with high affinity to mannose rich carbohydrate moieties of pathogens such as viral envelope proteins. CD209L initiates antigen internalisation and is therefore an important first step in antigen processing, presentation and the initiation of an immune response. Recently it has been shown that viruses such as HIV and HCV bind CD209L and are internalized into non-lysosomal compartments and subsequently transferred to infect target cells. For a recent review on CD209L please refer to Koppel *et al*. (2005) *Cell Microbiol* **7**(2):157-65.

FOR RESEARCH USE ONLY

human cell expressed CD209L- Fc^{HCX} Chimera

1D gel



2D gel

1D gel data

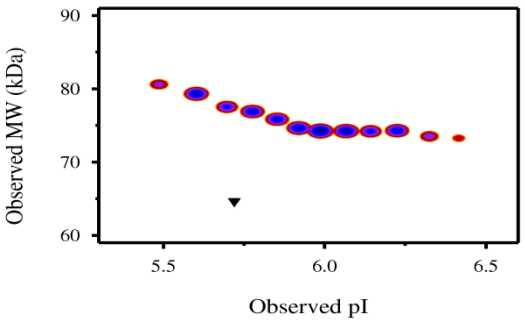
Lane 1 – MW markers; Lane 2 – CD209L-Fc^{HCX} Chimera; Lane 3 – CD209L-Fc^{HCX} Chimera treated with PNGase F to remove potential N-linked glycans. 10 µg protein loaded per lane. Deep Purple stained. Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Band in lane 3 at 35 kDa is PNGase F protein.

2D gel data

A sample of CD209L-Fc^{HCX} Chimera without carrier protein was reduced and alkylated. 40 µg protein was loaded, focused on a 3-10 IPG strip then run on a 4-20% Tris-HCl 2D gel. Spot train (Deep Purple™ stained) indicates presence of multiple glycoforms of CD209L-Fc^{HCX} Chimera. Spots within the spot train were cut from the gel and identified by protein mass fingerprinting as CD209L-Fc^{HCX} Chimera. 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 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.



Theoretical Sequence

HGTELPSPPSKLQVSKVPSSLSQEQSEQDAIYQNLTQLKAAVGELSEKSKLQEIYQELTQL
KAAVGELPEKSKLQEIYQELTRLKAAVGELPEKSKLQEIYQELTRLKAAVGELPEKSKLQEI
YQELTRLKAAVGELPEKSKLQEIYQELTELKAAVGELPEKSKLQEIYQELTQLKAAVGELPD
QSKQQQIYQELTDLKTAFERLCRHCPKDWTFFQGNCYFMSNSQRNWHDSVTACQEVRA
QLVVIKTAEEQNFLQLQTSRSNRFSWMGLSDLNQEGTWQWVDGSPLSPSFQRYWNSGE
PNNSGNEDCAEFSGSGWNDNRCDVDNYWICKKPAACFRDGIPKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCRVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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