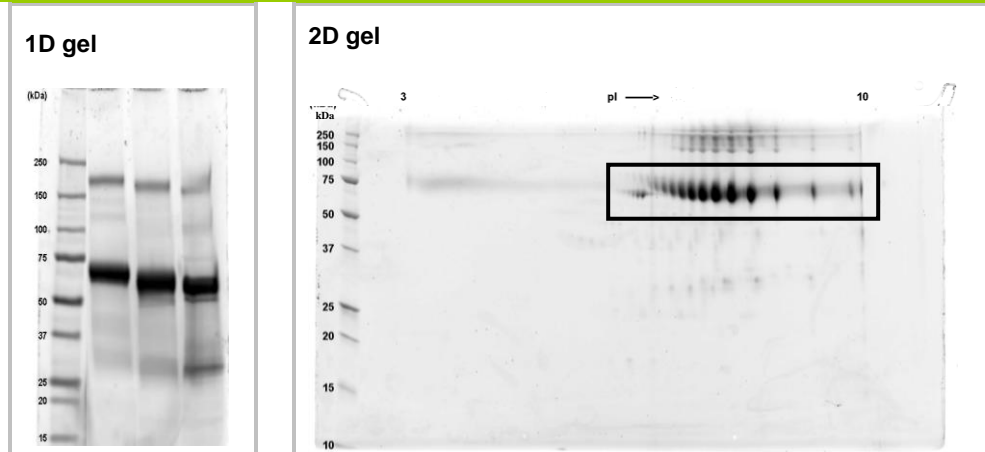


Siglec-3 – Fc Chimera^{HGX} Human Cell Expressed
Catalogue 9027

Source	DNA encoding the signal peptide and extracellular domain of human Siglec-3 (aa 1-259) chain was fused to the Fc region of human IgG1 (aa 93-330). The chimeric protein was expressed in modified human 293 cells.
Molecular Mass	Symansis Siglec-3 – Fc Chimera migrates as a broad band between 60 and 75 kDa on SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified Siglec-3 - Fc Chimera that has a predicted molecular mass of 54 kDa. Siglec-3 is also called CD33 or sialic acid binding Ig-like lectin 3.
pI	Symansis Siglec-3 – Fc Chimera separates into a number of glycoforms on 2D PAGE due to post-translational modifications, in particular glycosylation. The pI range is between 6.5 and 10. This compares with the unmodified Siglec-3 - Fc Chimera that has a predicted pI of 8.3.
%Carbohydrate	Symansis' purified Siglec-3 – Fc Chimera consists of 10-30% carbohydrate by weight.
Glycosylation	Siglec-3 – Fc Chimera has N-linked and may have 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	<p>Siglecs (or sialic acid-binding immunoglobulin superfamily lectins) are sialic acid-binding Ig-like lectins characterized by a homologous N-terminal V-set Ig-like domain and varying numbers of C2-set Ig-like domains. In humans 11 Siglecs have been described. Siglec-3 (CD33) is the smallest Siglec, with only 2 of these C2-set Ig domains. Siglec-3 is also called sialic acid binding Ig-like lectin 3 protein.</p> <p>Siglec-3 is a 67 kDa glycoprotein found predominantly on myeloid cells, including early myeloid precursors in the bone marrow and certain subsets of mature circulating myeloid cells. Siglec-3 has also been reported on dendritic cells, cord blood-derived natural killer (NK) cells, <i>in vitro</i> expanded T cells, and some biphenotypic leukaemia's. Siglec-3 was initially described as a marker for normal and leukemic myeloid progenitor cells, but has received renewed interest due to its demonstrated lectin activity for alpha 2-6 and alpha 2-3 sialylated N-linked oligosaccharides expressed on red blood cells (RBCs) and certain myeloid cells.</p> <p>For a recent review on Siglecs please refer to Varki <i>et al.</i> (2006) <i>Glycobiology</i> 16:1R-27R.</p>

human cell expressed Siglec-3 – Fc Chimera



1D gel data

Lane 1 – MW markers; Lane 2 – Siglec-3 – Fc Chimera; Lane 3 – Siglec-3 – Fc Chimera treated with PNGase F to remove potential N-linked glycans; Lane 4 – Siglec-3 – Fc Chimera treated with a glycosidase cocktail to remove potential N- and O-linked glycans. 10 µg of protein was loaded per lane; Gel was stained with Coomassie G250.

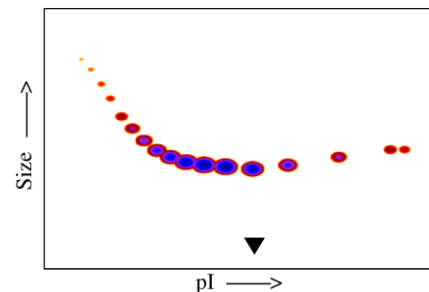
Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. Faint bands in lane 3 and lane 4 are glycosidase enzymes.

2D gel data

A sample of Siglec-3 – Fc 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. A spot train (Deep Purple™ stained) indicates the presence of multiple glycoforms of Siglec-3 - Fc Chimera. Spots within the spot train were cut from the gel and identified by protein mass fingerprinting as Siglec-3 – Fc 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

DPNFWLQVQESVTVQEGLCVLVPCTFFHPIPIYYDKNSPVHGYWFREGAIISGDSVPATNKLD
 QEVEETQGRFRLLGDPSRNNCSLSIVDARRRDNNGSYFFRMERGSTKYSYKSPQLSVHVTD
 LTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPIFSWLSAAPTS LGPRTHSSVLIITPRPQ
 DHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGDGSQKQETRAGVVHRSSNTK
 VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
 VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
 PVLDSDDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKLSLSLSPGK