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Product Data Sheet

Human Cell Expressed Adiponectin HCX™ Catalogue # 9510H

Source	A DNA sequence encoding the human Adiponectin protein sequence (containing the signal peptide sequence, and the mature Adiponectin sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis Adiponectin HCX™ migrates as a broad band between 25 and 35 kDa in SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified Adiponectin that has a predicted molecular mass of 24.5 kDa.
pI	Symansis Adiponectin HCX™ separates into a number of glycoforms with an observed pI between 4.5 and 8.0 on 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified Adiponectin that has a predicted pI of 5.5.
% Carbohydrate	Symansis purified Adiponectin HCX™ consists of 0 to 30% carbohydrate by weight.
Glycosylation	Symansis Adiponectin HCX™ contains 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 adiponectin HCX™ is typically 3-5 ug/ml as measured by its ability to inhibit M1 cell proliferation.
Background Information	Adiponectin is a member of the 'adipocytokines' that are cytokines that are expressed from adipose tissues. Its general functions include regulation of energy homeostasis, hematopoiesis, inflammation and immunity. Adiponectin also plays an important role in fat metabolism, feeding behaviour, insulin sensitivity and is a negative regulator of hematopoiesis and immune responses. Adiponectin has been shown to suppress the expression of a number of membrane bound proteins involved in the infiltration of cells to sites of inflammation, including, vascular cell adhesion molecule 1 (VCAM-1), E-Selectin, and intercellular adhesion molecule-1 (ICAM-1). Furthermore adiponectin suppresses TNF induced adhesion of monocytes to endothelial cells through a mechanism involving the suppression of TNF-alpha induced I-kappa-B-alpha phosphorylation. Adiponectin also inhibits phagocytosis and bacterial lipopolysaccharide-induced production of TNF-alpha in mature macrophages. Conversely, TNF-alpha reduces the expression and secretion of adiponectin in differentiating primary human pre-adipocytes. For a recent review of adiponectin refer to Tilg H and Moschen AR (2006) Nat Rev Immunol. 6(10):772-783.
Theoretical Sequence	ETTTQGPGVLLPLPKGACTGWMAGIPGHPGHNGAPGRDGRDGTPGEKGEKGDPGLIGPKGDIGETGV PGAEGPRGFPGIQGRKGEPGEGAYVYRSAFSVGLETYVTIPNMPIRFTKIFYNQQNHYDGSTGKFHCNI PGLYYFAYHITVYMKDVKVSLFKKDKAMLFTYDQYQENNVDQASGSVLLHLEVGDQVWLQVYGEGER NGLYADNDNDSTFTGFLLYHDTN

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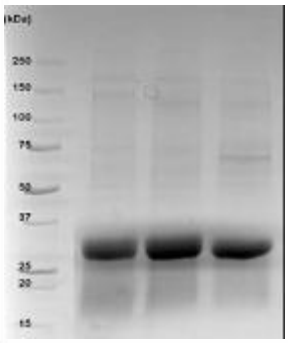
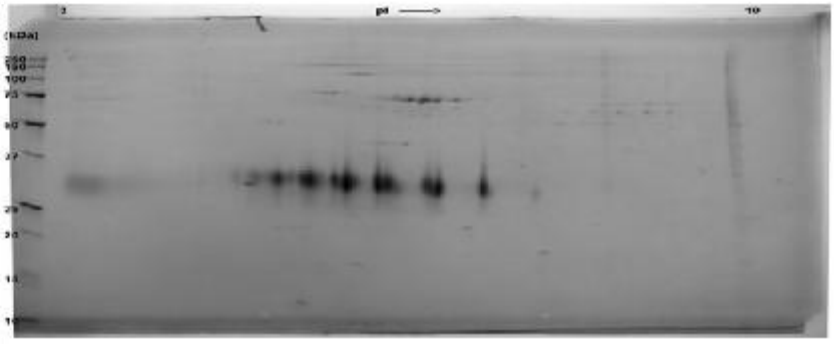
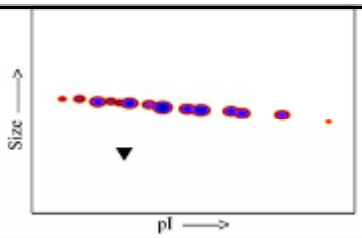
1D gel



2D Gel



1D gel data	Lane 1 – MW markers; Lane 2 – Adiponectin HCX™ ; Lane 3 – Adiponectin HCX™ treated with PNGase F to remove potential N-linked glycans; Lane 4 – Adiponectin HCX™ 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. No drop in MW was observed, therefore the protein does not have N-linked or mucin-type O-linked oligosaccharides. Additional bands in lane 3 and lane 4 are glycosidase enzymes. Note: the O-glycans present on Adiponectin are not linked to serine or threonine and are therefore not susceptible to the glycosidase cocktail used.
LC-MS data	Adiponectin HCX™ (2ug) was digested with trypsin then analysed by LC-MS in positive mode. Each of the four lysines at 47, 50, 59, and 83 was found to be hydroxylated and subsequently modified with a dihexose oligosaccharide. Similarly, proline 73 was also observed to be hyxdroylated. These results match those found by Wang *et al.* (2002) J Biol Chem 277:19521-19529 for endogenous human adiponectin purified from adipocytes.
2D gel data	A sample of Adiponectin HCX™ 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 load; Gel was stained using Deep Purple™. Spot train indicates presence of multiple isoforms of Adiponectin HCX™ . . Spots within the spot train were cut from the gel and identified as Adiponectin HCX™ by protein mass fingerprinting.
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 shown above.

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