Adiponectin

Adiponectin is an abundant protein hormone that belongs to a family of adipokines. It is expressed mostly by adipocytes and is an important regulator of lipid and glucose metabolism. Adiponectin is an insulin sensitizing hormone with anti-diabetic, anti-inflammatory and anti-atherogenic properties (1). It has been shown that the amount of adiponectin in blood reduces in cases of patients suffering from Type 2 diabetes mellitus or coronary artery diseases, or who are insulin-resistant (2-6).

Human adiponectin consists of 244 amino acid residues and has a distinct domain structure: It contains both collagen-like and globular C1q-like domains. Collagen-like parts of three adiponectin molecules can form a triple coiled coil structure very similar to that in collagen (7). C1q-like domains form a "head" of adiponectin globula (Figure 1) and share a considerable degree of structural similarity to complement component C1q.

In blood, adiponectin is found as trimers (low molecular weight form, LMW), hexamers (medium molecular weight form, MMW) and higher order multimers (high molecular weight form, HMW). HMW adiponectin consists of 12-18 monomers (8-9). It has been suggested that different oligomeric forms exist in blood as separate moieties and do not convert into one another (10). Adiponectin oligomers are capable of binding Ca^{2+} ions, which are thought to participate in the maintenance of conformational stability of adiponectin (11).

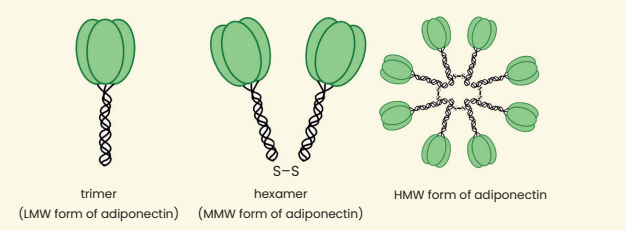

Figure 1.
Schematic representation of the oligomeric forms of adiponectin.

The concentration of total adiponectin in the blood is approximately 3-30 μg/ml, whereas the concentration of the closest structural homolog of adiponectin, C1q, is approximately 80-200 μg/ml. Therefore, it is critical that anti-adiponectin antibodies do not cross-react with human C1q (12). Some authors describe significant gender differences in adiponectin level in healthy adults. These differences could contribute to discrepancies in adiponectin concentrations reported by various authors. It has been suggested that the concentration of the HMW form of adiponectin or HMW/total adiponectin ratio correlates with insulin resistance and metabolic syndrome better than just the concentration of total adiponectin (13-14).

Reagents for the development of a reliable adiponectin assay

Hytest offers several anti-human adiponectin monoclonal antibodies (MAbs) and a native purified adiponectin that are suitable for the development of adiponectin specific immunoassays.

CLINICAL UTILITY
Type 2 diabetes

MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN ADIPONECTIN

We provide nine MAbs for the detection of human adiponectin. None of the MAbs cross-react with C1q in direct ELISA. C1q is the most abundant adiponectin homolog in blood. A detailed characterization of these adiponectin specific antibodies and antibody pairs was published in 2013 by our researchers (3).

Development of a sandwich immunoassay

All MAbs were tested in two-site combinations as capture or detection antibodies in sandwich ELISA with native adiponectin. We recommend six different two-site combinations for the development of sandwich immunoassays (see Table 1). These assays demonstrate high sensitivity and specificity to different oligomeric forms of adiponectin. A representative curve demonstrating detection of purified native adiponectin using the assay Adn279-Adn94 is provided in Figure 2.

All assays were tested with serial dilutions of normal human serum to evaluate the interaction of MAbs with native adiponectin in a complex environment. All assays demonstrated a steady decrease of signal correlating with the degree of serum dilution. The representative titration curve for the assay Adn94-Adn63 is provided in Figure 3.

Table 1.
Antibody pair recommendations.

Capture	Detection
Adn36	Adn27
Adn63	Adn94
Adn94	Adn63
Adn305cc	Adn279

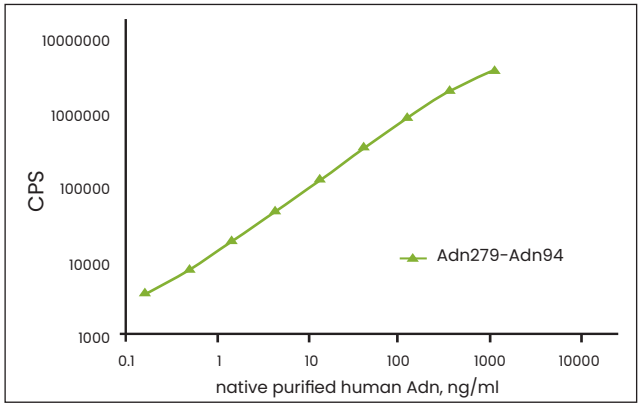

Figure 2.
Calibration curve for sandwich adiponectin immunoassay. *MAb Adn279 was used as a coating (1 µg/well), MAb Adn94 was labeled with stable Eu^{3+} chelate and was used as a detection (0.2 µg/well) antibody. Native adiponectin purified from human plasma was used as a calibrator.*

Effect of sample matrix and Ca^{2+}

Ca^{2+} participate in the formation of the adiponectin tertiary structure (11). Of the recommended two-site combinations, pair Adn36-Adn27 turned out to be sensitive to Ca^{2+} (not shown). This pair also shows a slightly different ability to detect adiponectin in serum or plasma samples (see Figure 4). Other assays do not demonstrate Ca^{2+}-dependence in the antigen recognition and react identically with adiponectin in serum or citrate plasma.

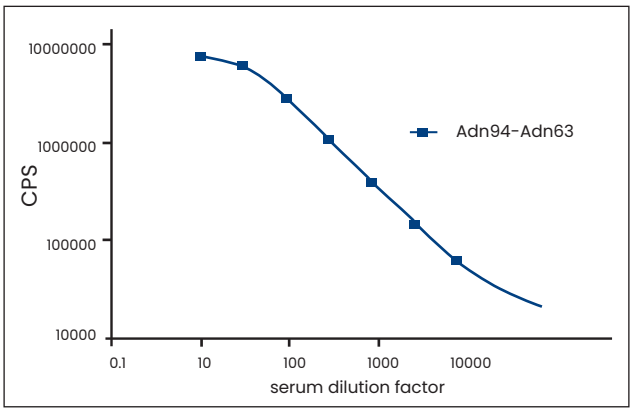

Figure 3.
Normal human serum titration curve in sandwich immunofluorescent assay. *Adn94 MAb was used as a coating antibody (1 µg/well), MAb Adn63 was used as a detection antibody (0.2 µg/well). Normal human serum, serially diluted with phosphate-buffered saline (10 mM K-phosphate, pH 7.4, 150 mM NaCl, 0.1% Tween-20) was used as an antigen.*

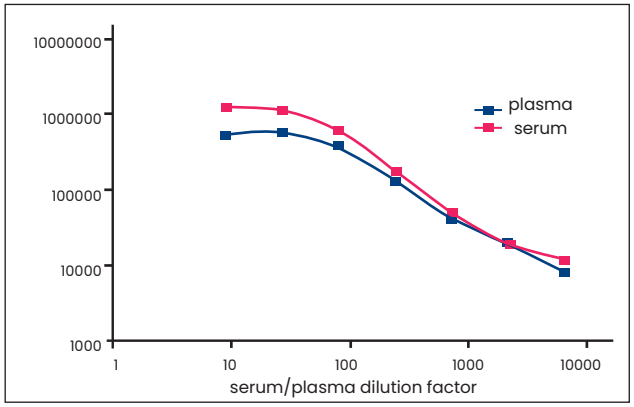

Figure 4.
Normal human serum or citrate plasma titration curves for MAb assay Adn36-Adn27. *Normal human pooled serum or citrate plasma, serially diluted with phosphate-buffered saline (10 mM K-phosphate, pH 7.4, 150 mM NaCl, 0.1% Tween-20) was used as an antigen.*

Western blotting

All MAbs were tested for their ability to recognize adiponectin in Western blotting. MAbs Adn23 and Adn63 recognized adiponectin transferred onto nitrocellulose membrane after SDS-PAGE in reducing conditions (see Figure 5).

Assays detecting total and LMW forms of human adiponectin

To establish an oligomer specificity of Hytest assays, serum proteins were separated according to their molecular masses by means of size-exclusion chromatography and immunoreactivity in fractions was measured. Assay Adn94-Adn63 recognizes all three adiponectin oligomeric forms (Figure 6).

NATIVE PURIFIED ADIPONECTIN

Native adiponectin purified from normal human plasma is the best calibrator for immunoassays. Native adiponectin was isolated from normal human plasma using a combination of chromatographic methods. Its purity is approximately 95%. Native purified adiponectin fully recovers its immunoreactivity after lyophilization and reconstitution by the addition of deionized water (see Figure 7).

Purified native adiponectin contains all three oligomeric forms of adiponectin (see Figure 8) and could therefore be used as a calibrator for all types of adiponectin assays: total adiponectin, HMW- or LMW-specific adiponectin.

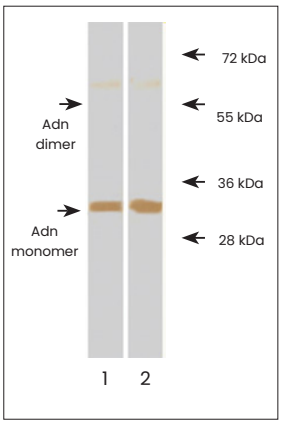

Figure 5.
Immunodetection of native adiponectin with anti-Adn MAbs in Western blotting after SDS-electrophoresis in reducing conditions. *40 ng of native purified adiponectin was loaded onto each track, nitrocellulose membrane was stained with 5 µg/ml of various anti-adiponectin MAbs in phosphate-buffered saline, containing 5% dry milk and 0.1% Tween-20. 1: Adn23, 2: Adn63, MW markers are marked by arrows.*

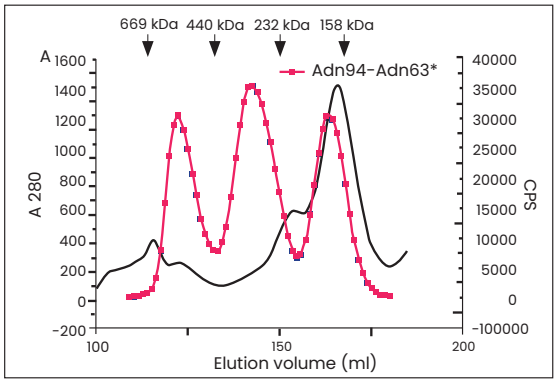

Figure 6.
Sandwich ELISA in protein fractions after size-exclusion chromatography, measured by capture-detection antibody pair Adn94-Adn63. *1 ml of normal human serum was applied onto the column. Positions of oligomeric forms of adiponectin and molecular weight markers are depicted in the picture. The black line presents the optical density detected at 280 nm.*

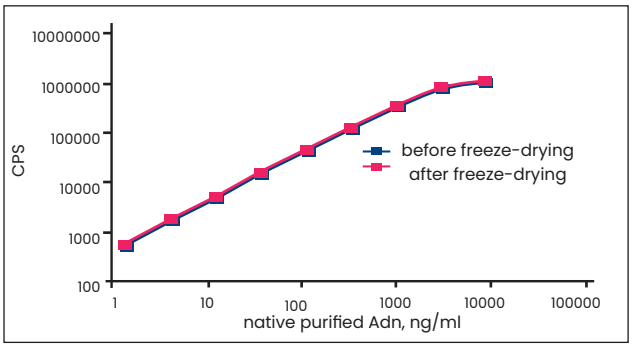

Figure 7.
Lyophilization does not affect immunological activity of native purified adiponectin measured by assay Adn94-Adn63.

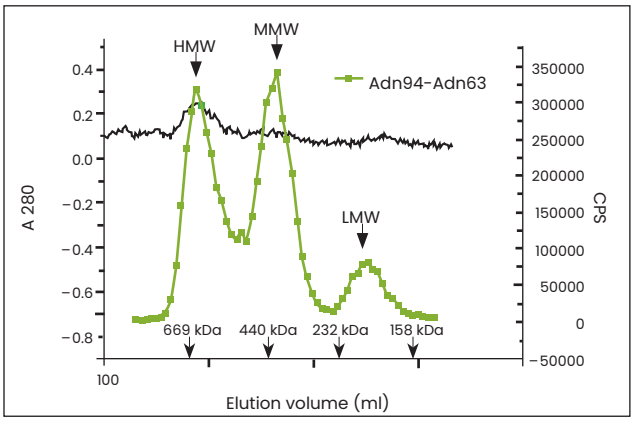

Figure 8.
Native purified adiponectin contains all oligomeric forms. *3 µg of adiponectin was applied onto a gel-filtration column and immunoreactivity in fractions was measured by the sandwich ELISA using Adn94 and Adn63 as capture and detection antibodies respectively. Molecular weight markers are depicted by arrows on the x-axis. The black curve represents the optical density measured at 280 nm.*

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Adiponectin, human	2AN6	Adn23	IgG2a	WB
		Adn27	IgG2a	EIA
		Adn36	IgG2a	EIA
		Adn63	IgG1	EIA, WB
		Adn94	IgG1	EIA
		Adn279	IgG1	EIA
		Adn305cc	IgG1	*In vitro*, EIA

ANTIGENS

Product name	Cat. #	Purity	Source
Adiponectin, human	8AN7	>95%	Pooled human plasma

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.

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