Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd

Adiponectin - marker for type 2 diabetes and cardiac disease

Reagents for the development of a reliable adiponectin assay

Advanced ImmunoChemical offers several anti-human adiponectin monoclonal antibodies and a native purified adiponectin that enable the development of an adiponectin specific immunoassays.

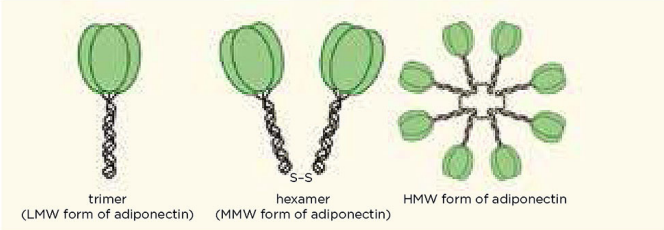

Figure 1. Schematic representation of the oligomeric forms of adiponectin. *Curr Med Chem 2012; 19 (32):5493-5500.*

Monoclonal antibodies specific to human adiponectin

Hybridoma clones were derived from hybridization of Sp2/0 myeloma cells with spleen cells of Balb/c mice immunized with either human recombinant adiponectin or native human adiponectin.
All antibodies were tested in direct ELISA for cross-reaction with C1q, which is the most abundant adiponectin homolog in blood. None of the selected MAbs showed any cross-reaction with human C1q.

Sandwich immunoassay

All MAbs were tested in two-site combinations as capture or detection antibodies in sandwich ELISA with native adiponectin. Seven two-site combinations were selected for the development of sandwich immunoassays on the basis of sensitivity and specificity to different oligomeric forms of adiponectin:

Adn20 - Adn23
Adn36 - Adn27
Adn94 - Adn63
Adn279 - Adn94
Adn214 - Adn27
Adn222 - Adn94
Adn305 - Adn279

A representative curve demonstrating detection of purified native adiponectin by the assay Adn279-Adn94 is shown on Fig. 2.

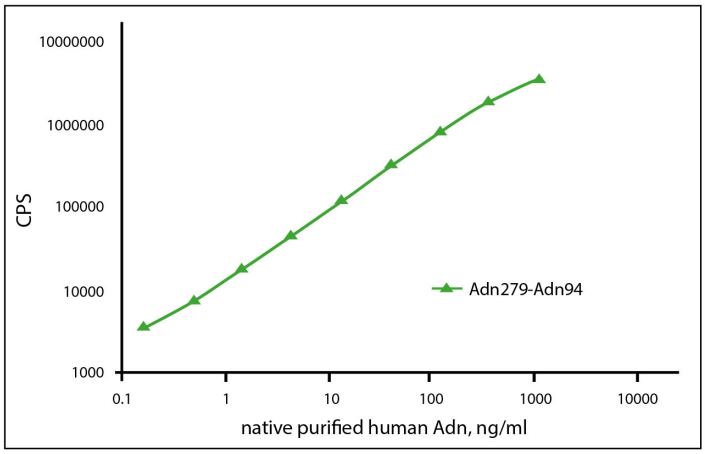

FIGURE 2. Calibration curve for sandwich adiponectin immunoassay. *MAb Adn279 was used as a coating (1 µg/well), MAb Adn94 was labeled with stable Eu3+ chelate and was used as a detection (0.2 µg/well) antibody. Native adiponectin purified from human plasma was used as a calibrator*

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 (capture antibody-detection antibody, respectively) is shown in Fig. 3.

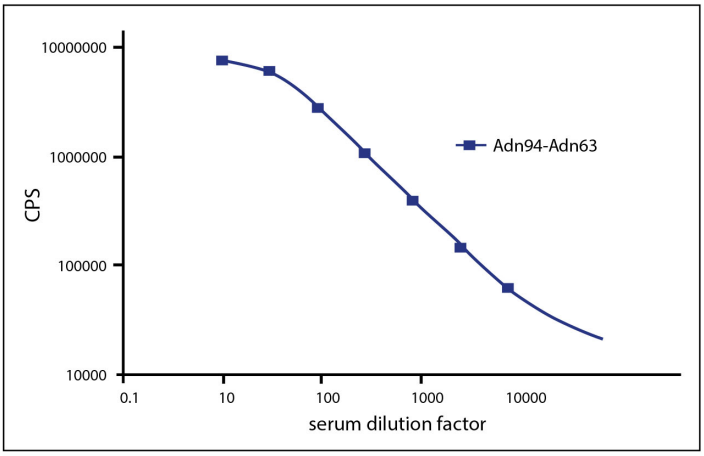

FIGURE 3. Normal human serum titration curve in sandwich immunofluorescent assay. *Adn94 MAb was used as a coating antibody (1 µg/well), MAb Adn63 was used as a detection antibody (0.2 µg/well). Normal human serum, serially diluted with phosphate- buffered saline (10 mM K-phosphate, pH 7.4, 150 mM NaCl,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

Assays Adn36-Adn27 and Adn20-Adn23 react differently with adiponectin in serum and citrate plasma (Fig. 4). Other MAbs two-site combinations (Adn94-Adn63, Adn279-Adn94, Adn214-Adn27, Adn222-Adn94, Adn305-Adn279) react identically with antigen in serum and plasma identically.

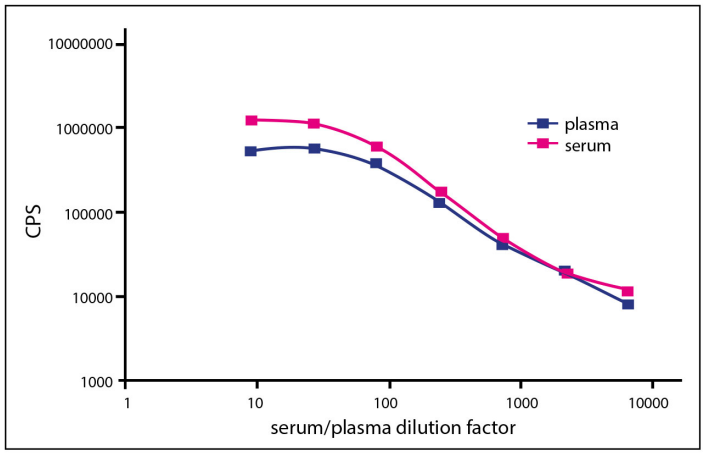

FIGURE 4. Normal human serum or citrate plasma titration curves for MAb assay Adn36-Adn27. *Normal human pooled serum or citrate plasma, serially diluted with phosphate-buffered saline (10 mM K-phosphate, pH 7.4, 150 mM NaCl, 0.1% Tween-20) was used as an antigen.*

Recognition of adiponectin by assays Adn20- Adn23 and Adn36-Adn27 in serum is Ca2+-sensitive (Fig. 5). Chelating of Ca2+ ions by EGTA leads to the rearrangements in adiponectin structure and changes in the interaction of one of the antibodies with the antigen. Other assays do not demonstrate Ca2+-dependence in the antigen recognition and react identically with adiponectin in serum or citrate plasma.

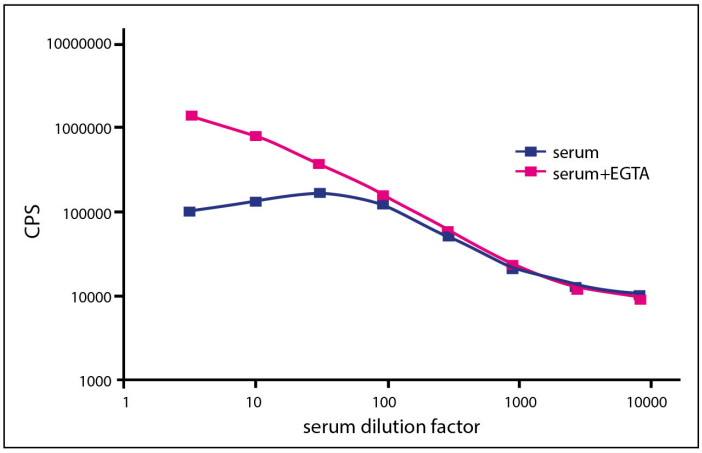

FIGURE 5. Serum titration curve for the assay Adn20-Adn23. *Pooled normal human serum was serially diluted with phosphate-buffered saline with EGTA or w/o EGTA (10 mM K-phosphate, pH 7.4, 150 mM NaCl, 0.1% Tween-20, 10 mM EGTA).*

Western blotting

All MAbs were tested on their ability to recognize adiponectin in Western blotting. Only six the of tested antibodies – MAbs Adn20, Adn23, Adn63, Adn214, Adn222, and Adn243 – reacted with adiponectin transferred onto nitrocellulose membrane after SDS-PAGE in reducing conditions (Fig. 6).

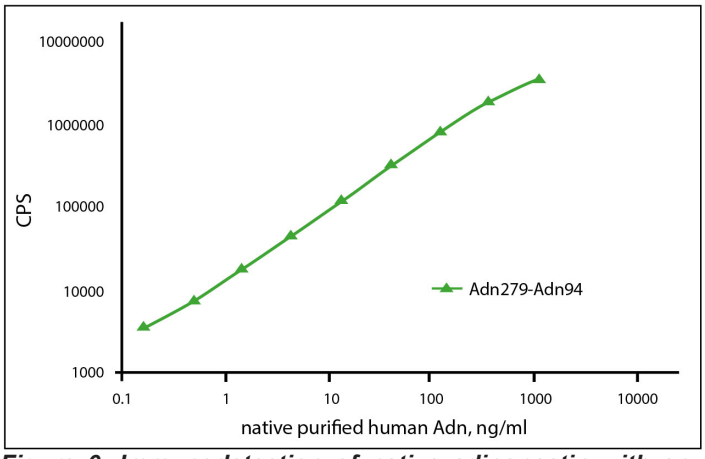

Figure 6. Immunodetection of native adiponectin with anti-Adn MAbs in Western blotting after SDS-electrophoresis in reducing conditions.
40 ng of native purified adiponectin was loaded onto each track, nitrocellulose membrane was stained with 5 µg/ml of various anti-adiponectin MAbs in phosphate-buffered saline, containing 5% dry milk and 0.1% Tween-20.
1: Adn20, 2: Adn23, 3: Adn63,
4: Adn214, 5: Adn222, 6: Adn243,
MW markers are marked by arrows.

Assays detecting total, HMW or LMW forms of human adiponectin

To establish an oligomer specificity of Advanced ImmunoChmical's assays, serum proteins were separated according to their molecular masses by means of size-exclusion chromatography and immunoreactivity in fractions was measured. The assay Adn20-Adn23 detects two oligomeric forms of adiponectin: mostly HMW and to a lesser extent, the MMW form (Fig. 7A). The assay Adn94-Adn63 recognizes all three Adn oligomeric forms - total adiponectin (Fig. 7B) and the assay Adn214-Adn27 reacts primarily with the LMW form of adiponectin (Fig. 7C).

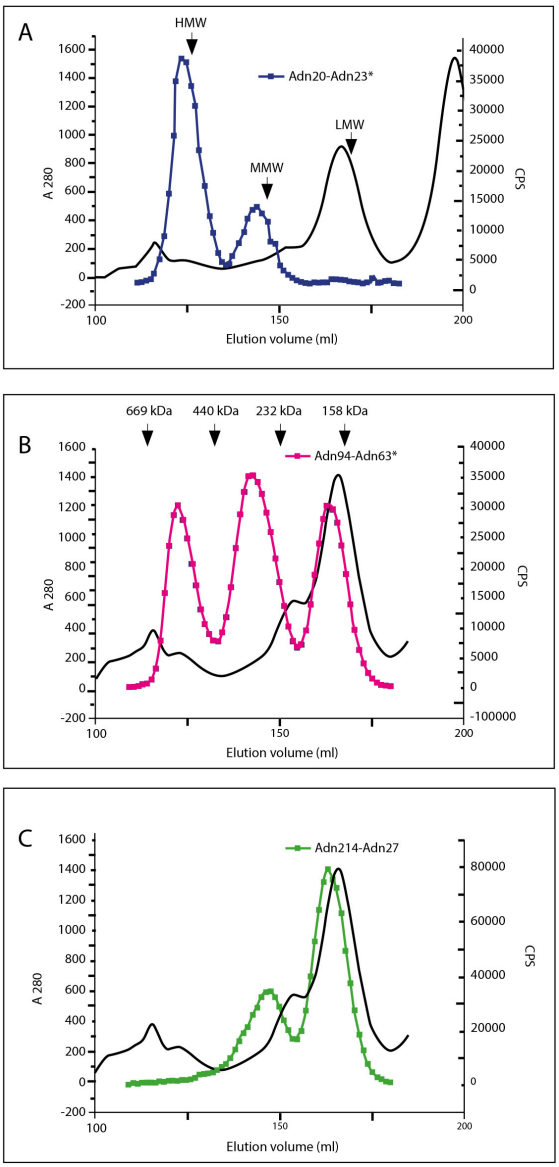

Figure 7. Sandwich ELISA in protein fractions after size-exclusion chromatography, measured by three different capture-detection antibody combinations. *(A) Adn20-Adn23, (B) Adn94-Adn63 and (C) Adn214-Adn27. 1 ml of normal human serum was applied onto the column. Positions of oligomeric forms of adiponectin and molecular weight markers are depicted in the picture. The black line presents the optical density detected at 280 nm.*

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 92%.

Native purified adiponectin fully recovers its immunoreactivity after lyophilization and reconstitution by the addition of deionized water (Fig. 8).

Purified native adiponectin contains all three oligomeric forms of Adn (Fig. 9) and can therefore serve as a calibrator for all types of Adn assays: total Adn, HMW- or LMW-specific.

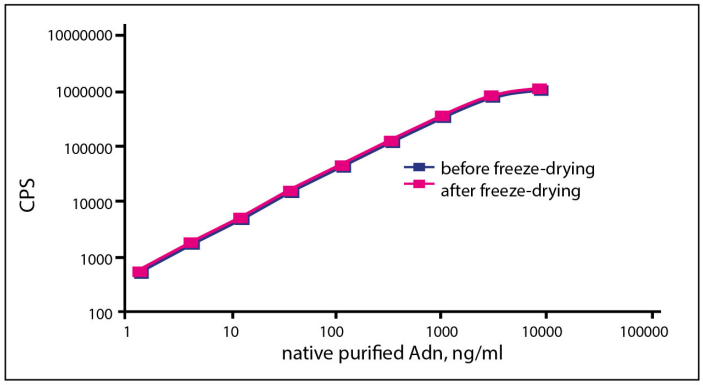

FIGURE 8. Lyophilization does not affect immunological activity of native purified adiponectin measured by assay Adn94-Adn63.

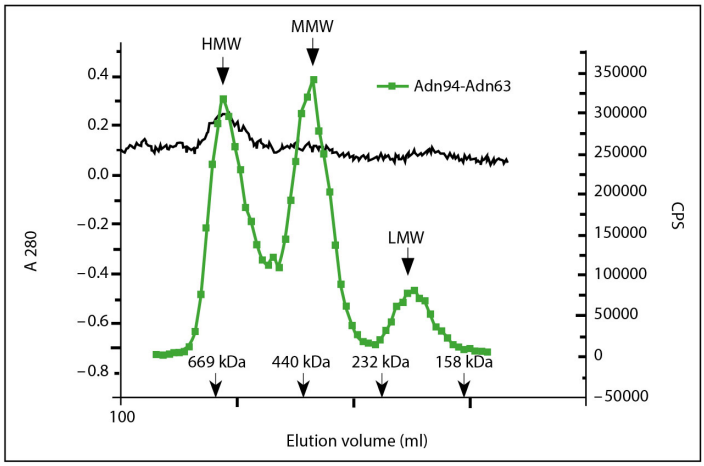

FIGURE 9. Native purified adiponectin contains all oligomeric forms. *3 µg of adiponectin was a1p50plied onto a gel-filtra-2t0i0on column and immunoreactivity in fractions was measured by the sandwich ELISA using Adn94 and Adn63 as capture and detection antibodies respectively. Molecular weight markers are depicted by arrows on the x-axis. The black curve represents the optical density measured at 280 nm.*

Ordering Information:

MONOCLONAL ANTIBODIES

Product	Cat #	MAb	Subclass	Remarks
Adiponectin, human	1-ADP	Adn20	IgG2a	EIA, WB
		Adn23	IgG2a	EIA, WB
		Adn27	IgG2a	EIA
		Adn36	IgG2a	EIA
		Adn63	IgG1	EIA, WB
		Adn94	IgG1	EIA
		Adn214	IgG1	EIA, WB
		Adn222	IgG1	EIA, WB
		Adn279	IgG1	EIA
		Adn305	IgG1	EIA

ANTIGEN

Product	Cat #	Purity	Source
Adiponectin, human	8-ADP	>95%	Pooled human plasma

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