Contents

Introduction

Adiponectin, also referred to as ACRP30, AdipoQ and gelatin-binding protein-28, is specifically and abundantly produced in adipocytes and accounts for about 0.01% of total plasma protein (1).

The primary structure of adiponectin consists of three domains, a short N-terminal region, a collagen-like domain, and a C-terminal globular domain which is structurally similar to complement C1q. Circulating adiponectin exists in several homo-oligomeric isoforms: a low molecular weigh trimers, a middle-molecular weight hexamers, and higher-molecular weight 12- to 18-mer adiponectins (2). In addition to oligomers, adiponectin can also be processed by proteolysis, and 16.5 kDa of a smaller globular domain fragment(gAcrp30) can be detected in plasma (3).

It is reported that adiponectin plays an important role in insulin resistance, type 2 diabetes, cardiovascular disease and the metabolic syndrome, which are linked to obesity. (4-7)

This adiponectin immunoassay kit is a 5 hour solid-phase ELISA designed to measure total (low, middle, and high molecular weight) human adiponectin in serum and plasma.

Assay Principle

This assay is based on the quantitative sandwich enzyme immunoassay technique in which two monoclonal antibodies are directed against separate antigenic determinants on the adiponectin. A monoclonal antibody specific for the adiponectin has been pre-coated onto a microplate. Standards, controls, and samples are pipetted into the wells and any adiponectin present is bound by the coated antibodies. After washing away any unbound substances, an biotinylated monoclonal antibody specific for adiponectin is added to the wells

Reagent Supplied

Component	Part No.	Quantity	Description
Microplate		12 strips of 8 wells	coated with anti-adiponectin monoclonal antibody; Ready for use
Assay Buffer		12ml	Ready for use
Dilution Buffer		125ml	for reconstituting standards and controls and diluting samples; Ready for use
Detection Antibody Solution		12ml	Biotinylated anti-human adiponectin monoclonal antibody; Ready for use
HRP Conjugate Solution		12ml	Streptavidin conjugated with horse redish peroxidase (HRP); Ready for use
20X Wash Buffer		60ml	20X concentrated wash buffer; Preparation
Standard		1 vial	Lyophilized standards; Concentration stated on vial label; Reconstitution
TMB Substrate Solution		12ml	TMB Peroxidase Substrate; Ready for use
Stop Solution		12ml	1M phosphoric acid; Ready for use
Plate sealers		3	
Instruction Manual		1	
Certificate of Analysis		1	

Other Supplies Required

- Test tubes diluting sample
- Microplate reader capable of measuring absorbance at 450nm, with the correction wavelength set at 550~650nm
- 20-1000µl pipettes and tips
- 50-200µl multi-channel pipettes and tips
- Redistilled water
- Wash device for microplates
- Beakers and cylinders for reagent preparation
- Absorbent materia for blotting the microplate (e.g., clean paper towels)

Warnings and Precautions

- For research use only.
- This kit contains reagents of human origin. These materials tested by a FDA approved method and found non-reactive for the presence of HbsAg, Hepatitis C, and antibody and antigen to HIV-1 and HIV-2. Nevertheless, these materials should be handled as potentially infectious, as no known test method can offer complete assurance of absence of infectious agents.
- Wear gloves and laboratory coats when handling immunodiagnostic materials and samples of human origin.

• Avoid contact with the Stop Solution and Color Reagent. Wear gloves and eye protection when handling these reagents.
• Reagents with different lot numbers should not be mixed or substituted.
• The kit should not be used beyond the expiration date on the kit label.

Sample Collection and Storage

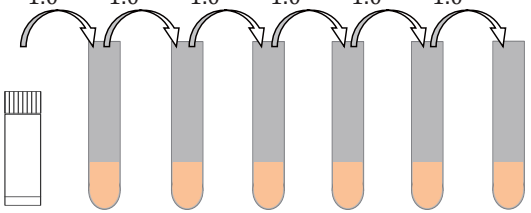
Serum
Collect blood, allow to clot for 30 minutes, and separate the serum by centrifugation for 15 minutes at 1000xg. Samples can be stored at -80°C. Avoid repeated freezing and thawing.

Plasma
Collect blood into tubes containing EDTA or heparin as anticoagulant, and separate the plasma fraction by centrifugation for 15 minutes at 1000xg within 30 minutes of collection. Samples can be stored at -80°C. Avoid repeated freezing and thawing.

Reagent and Sample Preparation

All reagents must be brought to room temperature before use.
Wash buffer : If crystal have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25ml of 20X Wash Buffer into 475ml of redistilled water to prepare 500ml of Wash Buffer(1X).
Standards : Reconstitute human adiponectin standards with Dilution Buffer according to the labels on the standard vial. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Perform 2-fold serial dilution from the reconstituted Standard stock as follows :
1.Label six 5ml polypropylene tubes.
2.Add the volume of 1X Dilution Buffer described in “initial vol.” in Fig.1 into each tube.
3.Transfer the indicated volume from previous Standard to next to produce a dilution series. Mix each tube completely before the next transfer.
4. The Standard #7 serves as the highest Standard. 1X Diluent only serves as the zero Standard (blank).

Tube #	stock (#7)	*#6*	*#5*	*#4*	*#3*	*#2*	*#1*	*blank*
initial vol. (ml)		1.0	1.0	1.0	1.0	1.0	1.0	
transfer vol. (ml)		1.0	1.0	1.0	1.0	1.0	1.0	
Conc. ***(ng/ml)***	***200***	***100***	***50***	***25***	***12.5***	***6.25***	***3.125***	***0***



<Fig.1> Standard preparation

Samples : Serum and plasma samples require at least 100-fold dilution. A suggested 100-fold dilution is 10µl sample + 990µl of Dilution Buffer.

Stability of Reconstituted and Opened Reagents

Component	Description
Microplate	For unused strips, completely reseal the bag using zip-seal. May be stored for up to 1 month at 2-8°C.*
Assay Buffer	May be stored for up to 1 month at 2-8°C.*
Dilution Buffer	May be stored for up to 1 month at 2-8°C.*
Detection Antibody Solution	May be stored for up to 1 month at 2-8°C.*
HRP Conjugate Solution	May be stored for up to 1 month at 2-8°C.*
20X Wash Buffer	May be stored for up to 1 month at 2-8°C.*
Standard	May be stored for up to 2 weeks at <-20°C after reconstitution.*
TMB Substrate Solution	May be stored for up to 1 month at 2-8°C.*
Stop Solution	May be stored for up to 1 month at 2-8°C.*
* Provided this is within the expiration date of the kit.	

Assay Procedure

All reagents and samples must be brought to room temperature before use.
Standards, controls and samples should be assayed in duplicate.

1. Prepare all reagents, standards, controls and samples as directed in the previous sections.
2. Prepare sufficient Microplate strips.
3. Add 100µl of Assay Buffer to each well.
4. Add 50µl of standards and samples per well. Cover with the plate sealer. Incubate for 2 horus at room temperature.
5. Wash 4 times with 360µl of Wash Buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent materials.
6. Add 100µl of Detection Antibody solution to each well. Cover with a new plate sealer. Incubate for 2 hours at room temperature.
7. Repeat wash step in step 5.
8. Add 100µl of HRP Conjugate solution to each well. Cover with a new plate sealer. Incubate for 30 minutes at room temperature.
9. Repeat wash step in step 5.
10. Add 100µl of TMB Substrate Solution to each well. Incubate for 20-30 minutes at room temperature. Protect from light.

11. Add 100µl of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

12. Determine the optical density of each well within 30 minutes, using a mciroplate reader set to 450nm. If wavelength correction is available, set to 540nm or 570nm. Readings made directly at 450nm without correction may be higher and less accurate.

Calculation of Results

- Average the duplicate readings for each standard, control, and sample and substract the average zero standard optical density.
- Plot the absorbance values for the standards against the standard concentration and draw the best curve (sigmoidal 4-parameter logistic equation is recommended).
- Read the concentration of the controls and samples from the standard curve.
- If the sample have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. The dilution factors of controls are 1.
- The assay will be considered accepted when all control values fall within the control range listed in Certificate of Analysis.

Limits of Assay

Results exceeding the highest standard should be repeated with more diluted samples with Dilution Buffer. The dilution factors need to be taken into consideration in calculating the concentration of adiponectin.

Performance Characteristics

Precision

Intra-Assay : Three samples of known concentration were tested twenty times on one plate.

Inter-Assay : Three samples of know concentration were tested in forty separate assays.

	Intra-Assay			Inter-Assay		
sample	1	2	3	1	2	3
n	20	20	20	40	40	40
mean (µg/ml)	3.99	9.48	12.08	4.01	10.92	18.28
standard deviation	0.16	0.47	0.71	0.25	0.40	0.72
CV	4.1%	5.0%	5.9%	6.3%	3.7%	3.9%

Spiking Recovery

The recovery of adiponectin spiked to levels throughout the range of the assay was evaluated.

sample	mean recovery	range
serum / plasma	95%	92-104%

Dilution Linearity

Samples containing and/or spiked with high concentrations of adiponectin were serially diluted with the Dilution Buffer before assay.

dilution factor	expected (µg/ml)	mean observed	range
2	9.5	102%	99-105%
4	4.75	101%	97-105%
8	2.375	99%	97-103%
16	1.188	103%	101-106%
32	0.594	89%	81-96%

Sensitivity

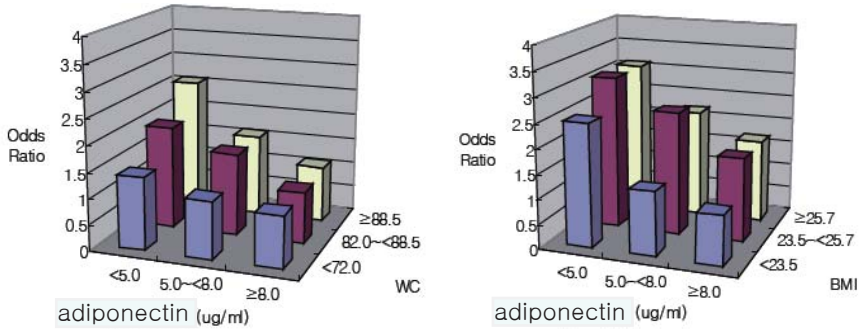
Twenty assays were evaluated and the detection limit was defined as blank + 2 standard deviation and determined to be 1.39 ng/ml

Reference Data

1. Sample Values: The following results were obtained when serum samples from 2,370 healthy persons were analysed.

	Men (n=1079)		Women (n=1291)	
	Mean	Standard deviation	Mean	Standard deviation
Adiponectin (μg/ml)	7.79	4.34	11.67	6.06
SBP (mmHg)	114.64	9.32	108.88	9.45
DBP (mmHg)	71.03	7.02	67.63	7.54
waist (cm)	80.55	5.51	70.52	4.82
FBS (mg/dL)	89.32	7.89	85.21	7.44
TG (mg/dL)	92.45	29.39	69.06	24.66
HDL (mg/dL)	54.52	10.08	65.01	10.81
SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBS: Fasting blood sugar; TG: Triglyceride; HDL: High density lipoprotein				

2. Combined effect of waist circumference (WC) and body mass index (BMI) with adiponectin on type 2 diabetes (T2DM) in 2648 men. (Ref. 8)



Reference

1. Arita Y. et al., Biochem. Biophys. Res. Commun. 257:79 (1999)
2. Tsao, T.S. et al., J. Biol. Chem., 277:29359 (2002)
3. Fruebis, J. et al., Proc. Natl. Acad. Sci. USA, 98:2005 (2001)
4. Nawrocki A.R. et al., J. Biol. Chem. 281:2654 (2006)
5. Tomas E. et al., Proc. Natl. Acad. Sci. USA 99:16309 (2002)
6. Spranger J. et al., Lancet 361:226 (2003)
7. Pischon T. et al., Jama 291:1730 (2004)
8. Yoon S.J. et al., Metabolism 57:853 (2008)