Human Adiponectin ELISA Kit

Enzyme Immunoassay for the quantification of human Adiponectin in serum and plasma

Catalog number: ARG80823

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INTRODUCTION

Adiponectin is a 30kDa protein which percentage in serum proteins is 0.01%. It is mainly synthesized by Adipocytes, but also muscle cells and hepatocytes have the ability to synthesize Adiponectin. Until now, IGF-I is the only known natural inductor of the synthesis. It consists of a Collagen-like N-terminal and a globular C-terminal domain. In vivo Adiponectin appears with different oligomers. Beside the trimer and ditrimer also high molecular multimers exist. Up to now two different receptors are known, both receptors are ubiquitary expressed, though the distribution in the tissues varies. The Adiponectin Receptor 1 (AdipoR1) is especially in muscle- and AdipoR2 in liver tissue synthesized.

The significance for the human organism is not clear until now. First studies show, that adiponectin correlates negatively with BMI and thus it could have relevance for the energy metabolism for example through the regulation of fatty acid oxidation. Beside the correlation with BMI, Adiponectin level is associated with the Insulin-Resistance and so also linked with Type II Diabetes. Adiponectin is associated also with glucose- und lipometabolism.

The formerly proposed diagnostic value of the high molecular weight form of adiponectin was not verified using a commercially available testsystem for the determination of HMW adiponectin. Blueher et al. clearly demonstrate that regarding the diagnosis of insulin resistance, measured by whole body glucose uptake below 40 µmol/kg*min, total adiponectin as determined with this

testkit, is with an area of 0.92 under the receiver-operating curve, of greater diagnostic value.

Furthermore it is involved in inflammatory processes and therewith it is of importance for appearance of arteriosclerosis and coronaritis, thus the determination of Adiponectin level in plasma could serve to estimate the risk of coronary disease. Beside this Adiponectin influences further physiological processes as for example the angiogenesis.

PRINCIPLE OF THE ASSAY

This assay employs the enzyme immunoassay technique. A highly specific Adiponectin antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Adiponectin present is bound by the immobilized antibody. After washing away any unbound substances, a mixture of biotin-conjugated antibody specific for Adiponectin and streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Adiponectin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Adiponectin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C.
Standards A-E (2, 10, 30, 70, 100 ng/ml)	5 vials	4°C, lyophilized
Control Serum KS1	1 vial	4°C, lyophilized
Control Serum KS2	1 vial	4°C, lyophilized
Antibody-POD-Conjugate (mixture of biotinylated anti-Adiponectin antibody and HRP-Streptavidin)	12 ml (ready to use)	4°C
Diluent Buffer	125 ml (ready to use)	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the Antibody-POD-Conjugate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. The diluted 1 X wash buffer is stable at 2-8°C for up to 4 weeks.
- Standards: Reconstituted in 750 µl (each) Dilution Buffer.
- Controls: Reconstituted in 500 µl (each) Dilution Buffer.

  Note: The reconstituted standards and Controls must be stored at –20°C for up to 4 weeks. For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay.
- Dilution of Samples and controls: Dilute sample and controls at 1:310 with Dilution Buffer.

  Example:

  1. Add 300 µL Dilution Buffer in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), then add 10 µL sample (dilution: 1:31).
  2. Add 900 µL Dilution Buffer in another PE-/PP-tube and 100 µL of the thoroughly mixed 1:31 diluted samples from step 1.
  3. After mixing, use 2×100 µL from this 1:310 diluted sample (from step 2) in the assay.
  4. Alternatively if you have the necessary technical equipment a one-step dilution of 1:301 is possible by adding 5 µL to 1.5 mL dilution buffer.
  5. Diluted Samples and controsl are stabile up to 1 hour at RT.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl Dilution Buffer (blank) in the first wells. Subsequently add 100 µl Standard or 100 µl of diluted Control Sera or diluted samples.
3. Cover wells and incubate for 60 minutes at RT on a microplate shaker at 350rpm.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of Antibody-POD-Conjugate into each well. Cover wells and incubate 30 minutes at RT on a microplate shaker at 350rpm.
6. Aspirate and wash each well as step 4.
7. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm within 30 min. And read at ≥ 590 nm as reference wavelength.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

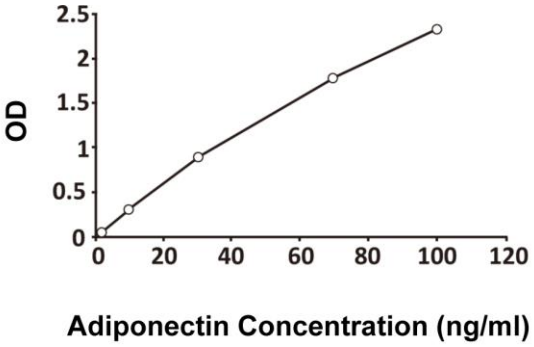
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. Quality criteria: For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance of standard E should be above 1.00. Samples, which yield higher absorbance values than Standard E, should be re-tested with a higher dilution.

6. If the dilution factor (1:310) is taken into account, the calculated adiponectin concentration from standard should be multiplied by 310 to get concentration of undiluted adiponectin.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Adiponectin range was <0.27 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.7% and inter-assay precision was 6.7%.

Specificity

Adiponectin exists in different oligomeric forms: the high, medium and low molecular weight form. Different numbers of the adiponectin monomer aggregate specifically to form a complex. The five different forms of human adiponectin are shown schematically. In parallel the results of a size-exclusion chromatography of human serum measured with this ELISA kit are shown this

ELISA kit detects all forms of Adiponectin present in human serum: the trimer at 65 kDa, the hexamer at 150 kDa and the high molecular weight forms of >280 kDa.

Recovery

101-116%