



Human Adiponectin ELISA

Catalog Number EA-0201

(For Research Use Only)

Introduction

Adiponectin, also called GBP-28, apM1, AdipoQ and Acrp30, is an adipose tissue-specific protein that circulates in human plasma at high levels. Plasma level of adiponectin has been reported to be significantly reduced in obese/diabetic mice and human (1). It is also decreased in patients with cardiovascular diseases, hypertension, or metabolic syndrome (2-5). On the contrary, a number of other human diseases such as chronic renal failure, type 1 diabetes and anorexia nervosa are associated with increased plasma adiponectin levels (6). Plasma adiponectin correlates negatively with glucose, insulin, triglyceride levels and body mass index, and positively with high-density lipoprotein-cholesterol levels and insulin-stimulated glucose disposal.

Principle of the assay

Adiponectin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse anti-human adiponectin antibody for immobilization on the microtiter wells and a biotinylated mouse anti-human adiponectin antibody along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the adiponectin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of stop solution changing the color to yellow. The concentration of adiponectin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
8x12 96-Well 12 strip Plate coated with mouse anti-human Adiponectin antibody	1	4°C
Biotin labeled mouse anti-human Adiponectin antibody	25µL	-20°C
Recombinant human Adiponectin standard (400ng/ml)	10µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	4°C

Material required but not provided

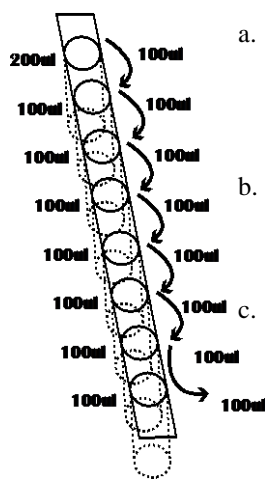
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
40ml 5x Assay wash buffer
160ml ddH₂O
- Dilute 100 times of Human recombinant Adiponectin (400ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions by adding 2ul Human Recombinant Adiponectin in 200ul 1X Diluent Buffer (See Step 2 in “Assay Procedure” for detailed instruction)
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 400 times of biotin labeled mouse anti-human Adiponectin antibody with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. See instruction and diagram below for standard preparation.



- a. Add 200ul 1X Diluent buffer to the 1st well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
- b. Add appropriate amount of protein recombinant (follow instruction in “Reagent Preparation”)
- c. Mix dilutions in 1st well and transfer 100ul from the 1st well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Add 100ul of sample per well and incubate for 1 hour at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200ul of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.

5. Add 100ul of diluted biotin-labeled mouse anti-human Adiponectin antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 100ul of substrate to each well and incubate for 10-30 minutes.
10. Add 50ul of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

References

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2. Hotta et. al. (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599.
3. Kumada et. al. (2003) Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 23:85–89.
4. Ouchi et. al. (2003) Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension* 42:231–234.
5. Trujillo and Scherer (2005) Adiponectin: journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J Intern Med* 257:167–175.
6. Diez and Iglesias (2003) The role of the novel adipocyte-derived hormone adiponectin in human disease. *European Journal of Endocrinology*, Vol 148, Issue 3, 293-300

Example of standard curve

