

# Human Adiponectin/Acrp30 Rabbit Polyclonal Antibody

Cat# CY-P1017

Amount: 50 µg (0.5 µg/µL)

Source Isotype	Clone Name	Applications*	Species Cross-Reactivity**
Rabbit IgG	-	WB, IP	H, M, R

## Introduction:

Adiponectin, also called GBP-28, apM1, AdipoQ and Acrp30, is a novel adipose tissue-specific protein that has structural homology to collagen VIII and X and complement factor C1q, and that circulates in human plasma at high levels. It is one of the physiologically active polypeptides secreted by adipose tissue, whose multiple functions have started to be understood in the last few Years. A reduction in adiponectin expression is associated with insulin resistance in some animal models. Administration of adiponectin has been accompanied by a reduction in plasma glucose and an increase in insulin sensitivity. In addition, thiazolidinediones, drugs that enhance insulin sensitivity through stimulation of the peroxisome proliferator-activated receptor-gamma, increase plasma adiponectin and mRNA levels in mice. On the other hand, this adipocyte protein seems to play a protective role in experimental models of vascular injury. In humans, adiponectin levels are inversely related to the degree of adiposity and positively associated with insulin sensitivity both in healthy subjects and in diabetic patients. Plasma adiponectin levels have been reported to be decreased in some insulin-resistant states, such as obesity and type 2 diabetes mellitus, and also in patients with coronary artery disease. On the contrary, chronic renal failure, type 1 diabetes and anorexia nervosa are associated with increased plasma adiponectin levels. Concentrations of plasma adiponectin have been shown to correlate negatively with glucose, insulin, triglyceride levels and body mass index, and positively with high-density lipoprotein-cholesterol levels and insulin-stimulated glucose disposal. Weight loss and therapy with thiazolidinediones increased endogenous adiponectin production in humans. Adiponectin increases insulin sensitivity by increasing tissue fat oxidation, resulting in reduced circulating fatty acid levels and reduced intracellular triglyceride contents in liver and muscle.

## Specificity/Sensitivity:

This antibody detects endogenous levels of adiponectin protein.

## Source/Purification:

This antibody is produced by immunizing rabbit with a recombinant human adiponectin produced by *E. coli*. IgG is purified by immunoaffinity chromatography.

## Recommended Antibody Dilutions:

Western blotting: 0.5-1 µg/mL, Immunoprecipitation: 1-2 µg /sample.

## Formulation & Storage:

Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

### \* Applications Key

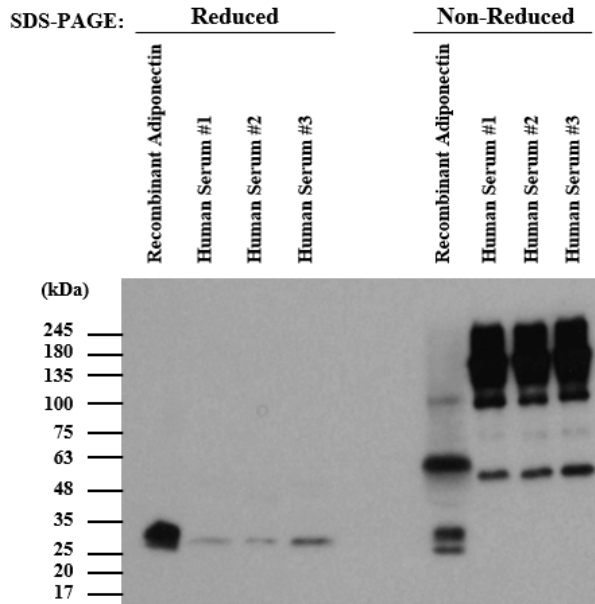
WB: Western blotting, IP: Immunoprecipitation, IHC: Immunohistochemistry, IC: Immunocytochemistry, E: ELISA, FC: Flow cytometry, FP: Fluorescence polarization assay, O: Others

### \*\* Species Cross-Reactivity Key

H: Human, M: Mouse, R: Rat, Hm: Hamster, Mk: Monkey, C: Chicken, O: Others

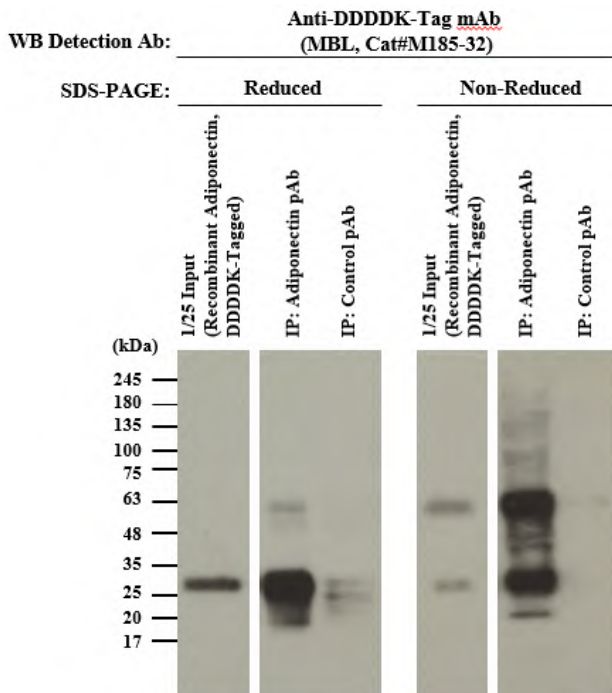
**Example of Test Results:**

Fig.1 Western blot analysis of human serum



Recombinant Adiponectin: Human, DDDDK-Tagged  
 Human Serum: 0.05µL/lane

Fig.2 Immunoprecipitation with recombinant adiponectin



**Protocol: Western blotting**

Note-1: Although we suggest to conduct experiments with cell lysate as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated and types of samples, and must be determined by the individual user.

Note-2: Prepare solutions with Milli-Q or equivalently purified water.

**I. Solutions and Reagents**

- SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 50 mM DTT, 0.01% bromophenol blue
- Transfer Buffer: 25 mM Tris-HCl (pH 8.5), 0.2 M glycine, 20% methanol
- 10X TBS (Tris-buffered saline): 0.5 M Tris-HCl (pH 7.6), 1.38 M NaCl
- Blocking Buffer: 1X TBS, 0.1% Tween®-20, 5% nonfat dry milk
- Antibody Dilution Buffer: 1X TBS, 0.1% Tween®-20, 5% blocking agent (e.g. BSA)
- TBS/T as a wash buffer: 1X TBS, 0.1% Tween®-20
- Blotting Membrane: PVDF membrane for protein blotting
- For chemiluminescent HRP detection:
  - Secondary antibody conjugated to horseradish peroxidase (HRP)
  - Enhanced chemiluminescence (ECL) reagent

**II. Protein Blotting**

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures, and wash cells with 1X PBS and aspirate.
3. Lyse cells by adding SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per Ø10 cm plate).
4. Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube.
5. Sonicate on ice for 10-15 seconds to shear DNA and reduce sample viscosity.
6. Heat samples to 95-100°C for 5 minutes.
7. Microcentrifuge for 5 minutes.
8. Load 20 µL of samples per lane onto SDS-PAGE gel (10 cm x 10 cm).
9. Perform electrophoresis and electrotransfer the gel to PVDF membrane by using Transfer Buffer.

**III. Membrane Blocking and Antibody Incubations**

1. After transfer, wash PVDF membrane with 25 mL of TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
4. Soak the membrane in 10 mL of the antibody appropriate diluted with Antibody Dilution Buffer.
5. Incubate with gentle agitation for appropriate time at room temperature or overnight at 4°C.
6. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
7. Soak the membrane in 10 mL of the secondary antibody appropriate diluted with Blocking Buffer.
8. Incubate with gentle agitation for 1 hour at room temperature.
9. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

**IV. Detection of Proteins**

1. Incubate membrane with 4 mL of ECL reagent with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution and wrap in plastic wrap. Do not let dry.
3. Expose to x-ray film or analyze using the chemiluminescence detecting apparatus.

**Protocol: Immunoprecipitation Followed by Western blotting**

Note-1: Although we suggest to conduct experiments with cell lysate as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated and types of samples, and must be determined by the individual user.

Note-2: Prepare solutions with Milli-Q or equivalently purified water.

**I. Solutions and Reagents**

- Cell Lysis Buffer: 20 mM Tris (pH 7.5), 135 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Glycerolphosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 µg/ml Leupeptin, adding PMSF to be the final 1mM just before use.
- Protein A Agarose Beads Solution: 50% slurry in PBS
- 3X SDS Sample Buffer: 187.5 mM Tris-HCl (pH 6.8), 6% SDS, 30% glycerol, 150 mM DTT, 0.03% bromophenol blue

**II. Preparing Cell Lysates**

1. Treat cells by adding fresh media containing regulators or drugs for desired time.
2. Aspirate media from cultures, and wash cells with 1X PBS and aspirate.
3. Lyse cells by adding ice-cold Cell Lysis Buffer (500 µL per Ø10 cm plate).
4. Incubate the plate on ice for 5 minutes.
5. Scrape the cells off the plate and transfer to microcentrifuge tubes.
6. Sonicate 4 times for 5 seconds each on ice.
7. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube.

**III. Immunoprecipitation**

1. Add the appropriate amount of an antibody for immunoprecipitation to 200 µL of each cell lysate.
  2. Incubate with gentle rocking overnight at 4°C.
  3. Add 20 µL of Protein A Agarose Beads Solution (50% slurry).
  4. Incubate with gentle rocking for 1 hour at 4°C.
  5. Wash pellets 3 times with 500 µL of ice-cold Cell Lysis Buffer.
  6. Resuspend the pellets with 20 µL of 3X SDS Sample Buffer.
  7. Heat the samples to 95-100°C for 5 minutes.
  8. Load 10-20 µL of samples per lane onto SDS-PAGE gel (10 cm x 10 cm).
  9. Perform electrophoresis and electrotransfer the gel to PVDF membrane by using Transfer Buffer.
- Follow “III. Membrane Blocking and Antibody Incubations” of the “Protocol: Western blotting” above.

**General References:**

1. Yamauchi T *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med.* 2001 Aug;7(8):941-6.
2. Weyer C *et al.* Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab.* 2001 May;86(5):1930-5.
3. Maeda N *et al.* PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes.* 2001 Sep;50(9):2094-9.
4. Yang WS *et al.* Synthetic peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. *Diabetes Care.* 2002 Feb;25(2):376-80.
5. Combs TP *et al.* Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. *Endocrinology.* 2002 Mar;143(3):998-1007.

**Related Products:**

- Rat Adiponectin ELISA Kit: CY-8049
- Human Adiponectin ELISA Ki: CY-8050
- Mouse Adiponectin ELISA Kit: CY-8051
- Dog Adiponectin ELISA Kit: CY-8052
- Human Adiponectin Rabbit Polyclonal antibody: Cat# CY-P1017
- Mouse Adiponectin Rabbit Polyclonal antibody: Cat# CY-P1018
- Human ANGPTL3 Rabbit Polyclonal antibody: Cat# CY-P1019
- Human ANGPTL4 Rabbit Polyclonal antibody: Cat# CY-P1021
- Mouse ANGPTL4 Rabbit Polyclonal antibody: Cat# CY-P1022
- Human Adiponectin (17-36 aa) Rabbit Polyclonal antibody: Cat# CY-P1031

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