Human Adiponectin/Acrp30 Rabbit Polyclonal Antibody

Cat# CY-P1017

50 µg (0.5 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB, IP	H, M, R	29 kDa	Rabbit IgG

Background

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: Human Adiponectin Antibody detects endogenous levels of Adiponectin protein.

Source/Purification: Polyclonal 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.

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

Applications Key: WB: Western **IP:** Immunoprecipitation **IHC:** Immunohistochemistry **IC:** Immunocytochemistry **F:** Flow cytometry **E:** ELISA **FP:** Fluorescence Polarization assay
Species Cross-Reactivity Key: H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Mi:** mink **C:** chicken **X:** *Xenopus* **Z:** zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

General References:

1. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* **7**: 941–946, 2001

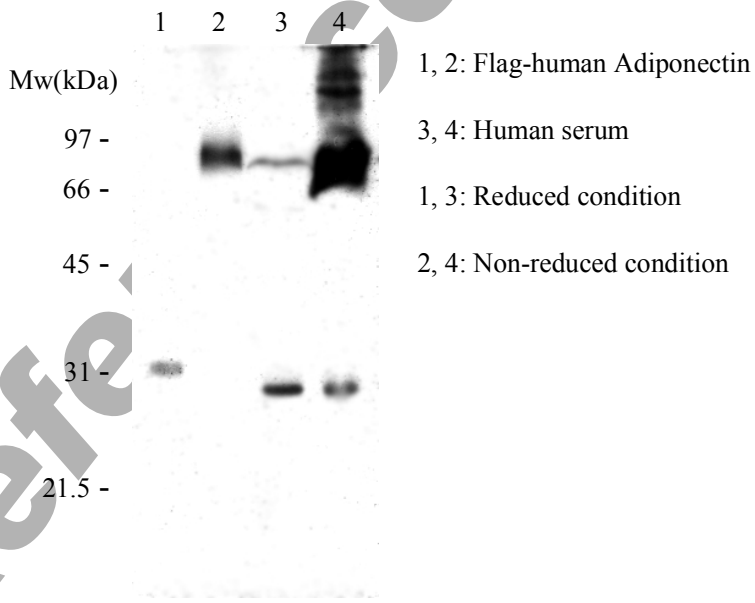
2. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectimia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* **86**: 1930–1935, 2001

3. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y: PPAR gamma ligands increase expression and plasma concentrations of adiponectin an adipose derived protein. *Diabetes* **50**: 2094–2099, 2001

4. Yang WS, Jeng CY, Wu TJ, Tanaka S, Funahashi T, Matsuzawa Y, Wang JP, Chen CL, Tai TY, Chuang LM: Synthetic peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. *Diabetes Care* **25**: 376–380, 2002

5. Combs TP, Wagner JA, Berger J, Doebber T, Wang WJ, Zhang BB, Tanen M, Berg AH, O'Rahilly S, Savage DB, Chatterjee K, Weiss S, Larson PJ, Gottesdiener KM, Gertz BJ, Charron MJ, Scherer PE, Moller DE: Induction of adipocyte complement related protein of 30 kilodaltons by PPAR-gamma agonists: a potential mechanism of insulin sensitization. *Endocrinology* **143**: 998–1007, 2002

Fig.1 Western blot analysis of Human Adiponectin



Western Immunoblotting Protocol

Solutions and Reagents

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

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit IgG antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL 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. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

Related Products

*** 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**

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