Porcine Adiponectin Immunoassay

Catalog Number: SEKP-0041

For the quantitative determination of Porcine adiponectin concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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BACKGROUND

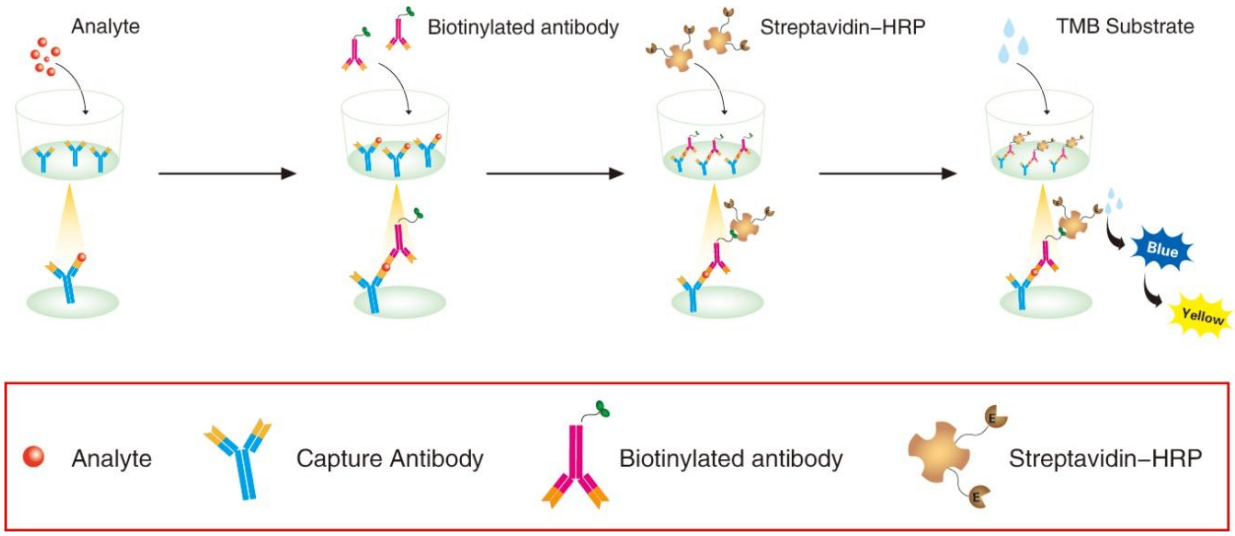
Adiponectin (also referred to as GBP-28, apM1, AdipoQ and Acrp30) is a protein hormone which in humans is encoded by the *ADIPOQ* gene. It is involved in regulating glucose levels as well as fatty acid breakdown. Adiponectin is a 244-amino-acid-long polypeptide. There are four distinct regions of adiponectin. The first is a short signal sequence that targets the hormone for secretion outside the cell; next is a short region that varies between species; the third is a 65-amino acid region with similarity to collagenous proteins; the last is a globular domain. Adiponectin modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation.Adiponectin is exclusively secreted from adipose tissue (and also from the placenta in pregnancy) into the bloodstream and is very abundant in plasma relative to many hormones. Levels of the hormone are inversely correlated with body fat percentage in adults. The hormone plays a role in the suppression of the metabolic derangements that may result in type 2 diabetes, obesity, atherosclerosis, non-alcoholic fatty liver disease (NAFLD) and an independent risk factor for metabolic syndrome. Adiponectin in combination with leptin has been shown to completely reverse insulin resistance in mice.

Adiponectin is secreted into the bloodstream where it accounts for approximately 0.01% of all plasma protein at around 5-10 μg/mL. Levels of adiponectin are reduced in diabetics compared to non-diabetics. Weight reduction significantly increases circulating levels. Recent studies showed that the high-molecular weight form may be the most biologically active form regarding glucose homeostasis.High-molecular-weight adiponectin was further found to be associated with a lower risk of diabetes with similar magnitude of association as total adiponectin. However, coronary artery disease has been found to be positively associated with high molecular weight adiponectin, but not with low molecular weight adiponectin. Adiponectin exerts some of its weight reduction effects via the brain. This is similar to the action of leptin, but the two hormones perform complementary actions, and can have synergistic effects.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for adiponectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any adiponectin present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for adiponectin is added to detect the captured adiponectin protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram：



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,5000pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
lyophilized Biotin-Conjugated antibody	1 vials	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8°C** for six months
Standard /sample Diluent	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent	1 bottle	Store at 2-8°C** for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8°C** for six months
Substrate Solution	1 bottle	Store at 2-8°C** for six months
Stop Solution	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

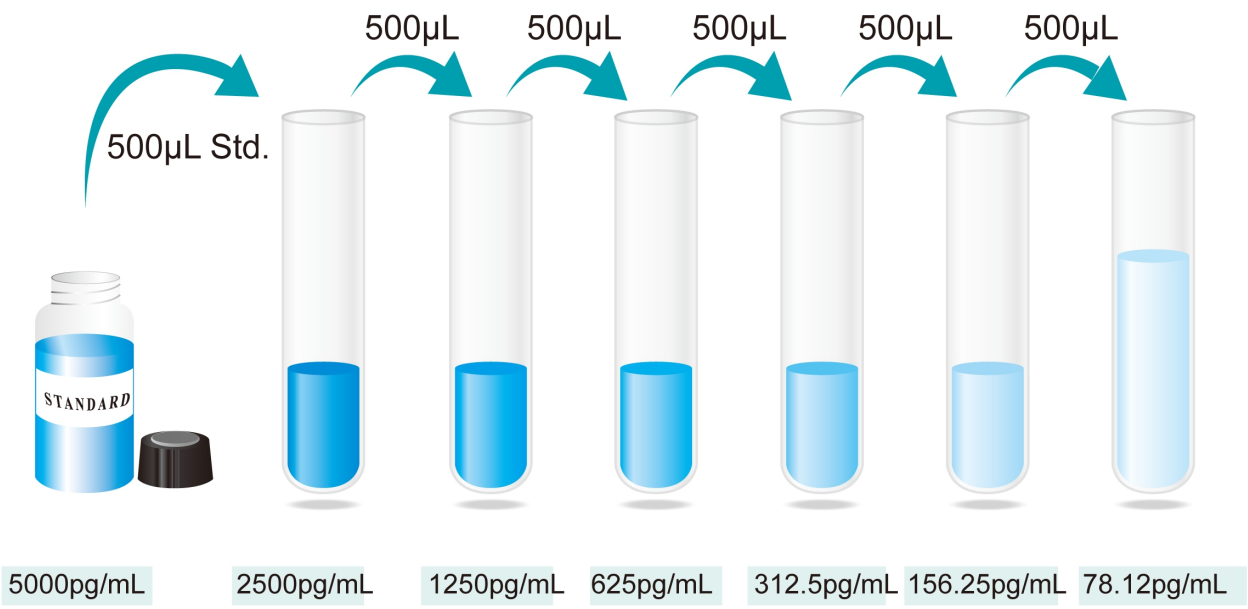
Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: If Adiponectin exceeds the upper limit of the detection, the sample needs to be diluted with Standard /Sample Diluent. The dilution factor must be used for calculation of the concentration.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.

2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard\Sample(2 vials)** - Porcine adiponectin Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of **Standard /Sample Diluent**. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.Pipette 500μL of **Standard /Sample Diluent** into 2500pg/ml tube and the remaining tubes. Use the stock solution of 5000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly(vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 5000 pg/mL standard serves as the high standard. The **Standard /Sample Diluent** serves as the zero standard (0 pg/mL).



Preparation of Porcine adiponectin standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-Porcine adiponectin antibody(1 vials)** - The lyophilized Detection Antibody should be stored at 4℃ to -20℃ in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110 µL of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 100µL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used. make a **1:100** dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP(120µL)** - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120 µL HRP Conjugate sufficient for a 96-well plate.Make **1:100** dilutions in Reagent Diluent. If the entire 96-well plate is used, add 100 ul of HRP Conjugate to 10 mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4° C for up to 6 months. DO NOT FREEZE.

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

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Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩

Add 100µl working solution of porcine-Conjugate anti-Porcine adiponectin antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

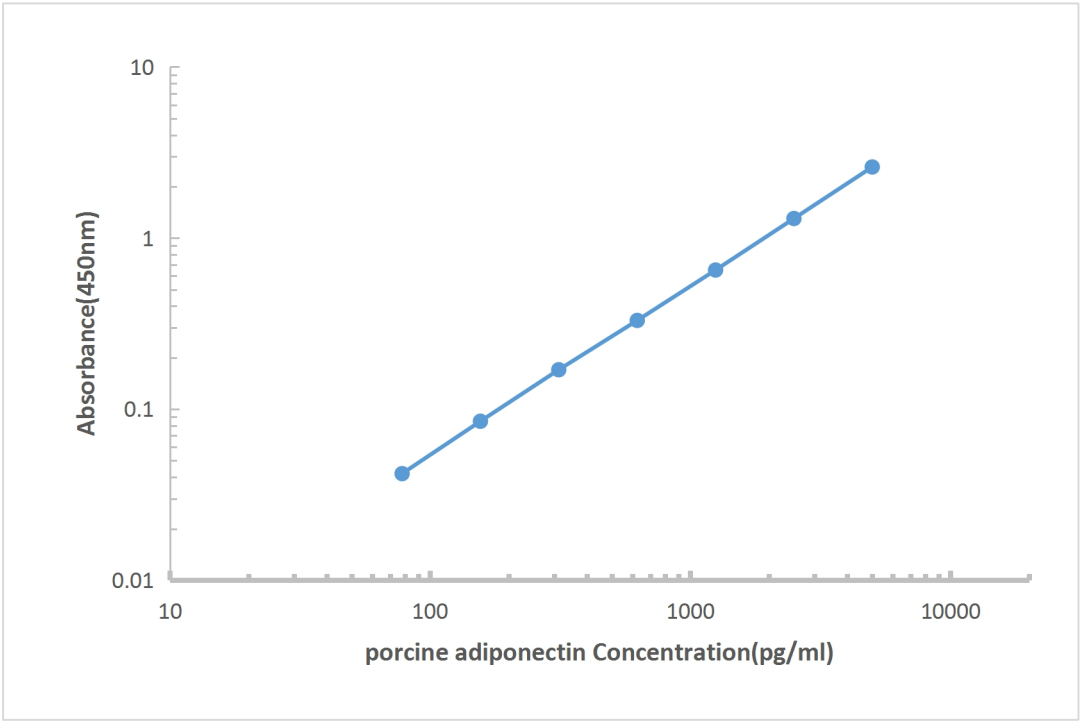
Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the adiponectin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the adiponectin ELISA

Std (pg/mL)	O.D.1	O.D.2	Averag	Correct
0	0. 053	0. 0454	0. 049	---
78.125	0. 097	0. 099	0. 098	0. 048
156.25	0. 187	0. 179	0. 183	0. 133
312.5	0. 293	0. 276	0. 284	0. 235
625	0. 479	0. 453	0. 466	0. 416
1250	0. 736	0. 759	0. 747	0. 698
2500	1. 254	1. 281	1. 267	1. 218
5000	2. 065	2. 083	2. 074	2. 024



Representative standard curve for adiponectin ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 15 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Porcine adiponectin. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

ApoAI, BMP1, BMP2, BMP4, BMP5, BMP7, CCL2, CCL4, CCL5, CRP, HGF, HSP27, IL-1β, IL1R1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17C, IL-21, IL-23, IFNβ, IFN-γ, IGF1, MMP-2, MMP-9, PDGF, serpin E1, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TLR9, TNF-α, TNF RI, TNF RII, sIL2R, sIL6R, VEGF, VEGF R1

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REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of adiponectin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of adiponectin in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	96	88-103
Cell culture supernatants	98	90-107

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of adiponectin in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	92	108
	Range (%)	83-101	98-117
1:4	Average% of Expected	95	110
	Range (%)	89-103	99-117

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