Quantikine® ELISA

Mouse Adiponectin/Acrp30 Immunoassay

Catalog Number MRP300
SMRP300
PMRP300

For the quantitative determination of mouse Adiponectin concentrations in cell culture supernates, tissue homogenates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Adiponectin, alternatively named Adipocyte Complement-Related Protein of 30 kDa (Acrp30), adipoQ, adipose most abundant gene transcript 1 (apM1), and gelatin-binding protein of 28 kDa (GBP28), shares structural similarity with complement factor C1q and is a member of the family of defense collagens. Adiponectin is secreted exclusively by differentiated adipocytes and circulates at high concentrations (1-7). Mouse Adiponectin cDNA encodes a 247 amino acid (aa) precursor protein with a putative 17 aa signal sequence. Mature mouse Adiponectin shares 86% and 93% aa sequence identity with human and rat Adiponectin, respectively (3, 8, 9). Adiponectin has a modular structure comprising an N-terminal collagenous domain with multiple collagen triple helix repeats, followed by a C-terminal C1q-like globular domain. The globular domain has similar folding topology with TNF-α and assembles into homotrimers. Adiponectin circulates as a homotrimer, hexamer, and higher-order multimer (1, 2, 4-7). Within each 90 kDa homotrimer, two monomers are disulfide-linked while the third subunit is non-covalently associated. For a hexamer, one non-covalently associated trimer subunit covalently links to a second non-covalently associated trimer subunit to create a 180 kDa complex (1, 7). Higher order 300-400 kDa multimers are known to exist, but how they form is unknown. A truncated trimeric Adiponectin containing only the globular domain (gAdiponectin/gAcrp30) can be generated by proteolytic cleavage. The gAdiponectin, as well as all oligomeric forms of the full length Adiponectin are detected in serum. Their relative ratio varies depending on gender and physiological conditions (1-7,10).

Different isoforms of Adiponectin have been shown to activate different signal transduction pathways. Hexameric and HMW isoforms of Adiponectin activate NF-κB. In contrast, trimeric Adiponectin and gAdiponectin signal through AMP-activated protein kinase. Conflicting biological activities have been reported for the various isoforms. It is possible that these contradictory activities result from the use of non-homogeneous isoform preparations (4, 7, 10-13).

Adiponectin is an anti-diabetic and anti-atherogenic hormone that plays important roles in the regulation of lipid and glucose metabolism. Its mode of action is opposite that of TNF-α. Adiponectin and TNF-α reciprocally regulate each other's expression and function (14, 15). Whereas TNF-α blocks glucose uptake and decreases fatty-acid clearance in skeletal muscle, Adiponectin reverses these TNF-α-mediated effects and enhances insulin sensitivity by activating glucose uptake and accelerating fatty acid oxidation and clearance (16). Adiponectin also inhibits the rate of endogenous hepatic glucose production to lower blood glucose levels (17). On the endothelium, Adiponectin reverses the effects of TNF-α-induced expression of adhesion molecules which cause abnormal leukocyte adhesion and excess inflammatory response (18, 19). Adiponectin also has a role in hematopoiesis and immune responses. It inhibits the growth of myelomonocytic progenitors and suppresses the phagocytic activity of macrophages. Suppression of macrophage phagocytosis by Adiponectin has been shown to be mediated by the complement receptor C1qRp (20).

Two seven membrane-spanning Adiponectin receptors, designated AdipoR1 and AdipoR2, have been identified. Both receptors are structurally and functionally distinct from typical G-protein coupled receptors. AdipoR1 is expressed predominantly in muscle and functions as a high-affinity receptor for gAdiponectin, but a very low-affinity receptor for the full length Adiponectin. AdipoR2 binds both the full length and globulin domain with intermediate affinity and is expressed primarily in liver. It has not been determined if the different oligomeric full length isoforms have different affinities for the two receptors (21).

The Quantikine Mouse Adiponectin Immunoassay is a 4.5 hour solid phase ELISA designed to measure full-length mouse Adiponectin levels in cell culture supernates, tissue homogenates, serum, and plasma. It contains NS0-expressed recombinant mouse Adiponectin and antibodies raised against the recombinant protein. Results obtained for naturally occurring mouse Adiponectin showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values of natural mouse Adiponectin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse Adiponectin has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse Adiponectin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Adiponectin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse Adiponectin bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # MRP300	CATALOG # SMRP300	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse Adiponectin Microplate	892552	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse Adiponectin.	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 °C.*
Mouse Adiponectin Conjugate	892553	1 vial	6 vials	12 mL/vial of a polyclonal antibody against mouse Adiponectin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse Adiponectin Standard	892554	1 vial	6 vials	50 ng/vial of recombinant mouse Adiponectin in a buffered protein base with preservatives; lyophilized.	
Mouse Adiponectin Control	892555	1 vial	6 vials	Recombinant mouse Adiponectin in a buffered protein base with preservatives; lyophilized. The concentration range of recombinant mouse Adiponectin after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1W	895038	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	2 vials	12 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

MRP300 contains sufficient materials to run ELISA on one 96 well plate.
SMRP300 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PMRP300). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates - Organs from 2-5 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized in 5-10 mL of PBS in a tissue homogenizer, and stored at ≤ -20 °C overnight. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g to remove particulate.Fat tissue collected from 3 female mice was homogenized in 5-10 mL of PBS and stored at ≤ -20 °C overnight. Homogenates were centrifuged for 5 minutes at 5000 x g.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples generally require at least a 2000-fold dilution into Calibrator Diluent RD5-26 (1X) prior to assay. A suggested 2000-fold dilution is achieved by creating a 100-fold dilution of 10 µL of sample + 990 µL of Calibrator Diluent RD5-26 (1X). Further dilute 20-fold with 10 µL of the 100-fold diluted sample + 190 µL of Calibrator Diluent RD5-26 (1X).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Adiponectin Control - Reconstitute the Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

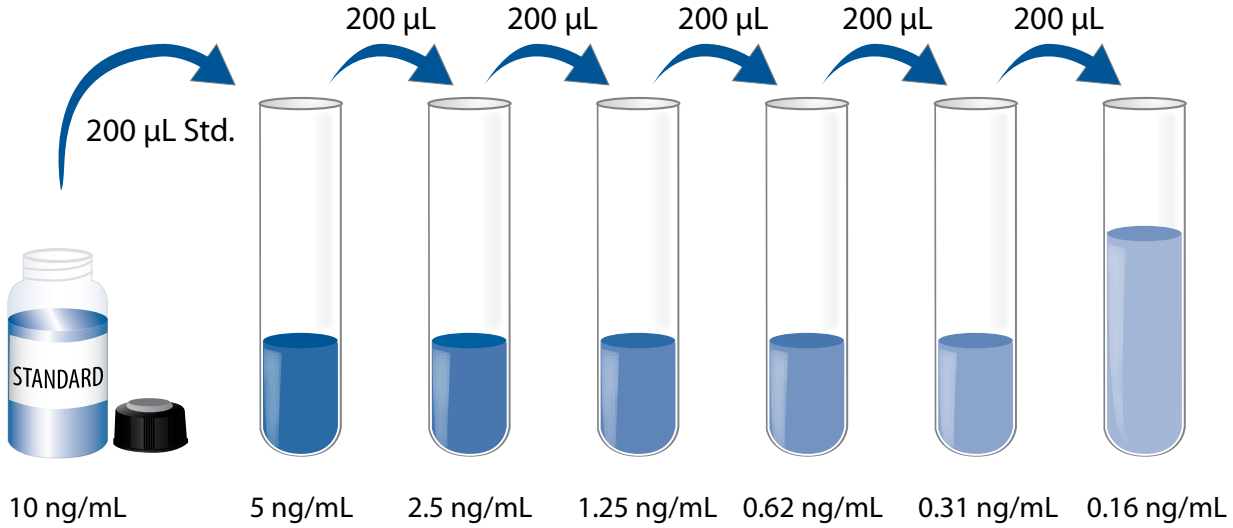
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (1X) - Dilute 20 mL of Calibrator Diluent RD5-26 Concentrate into 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (1X).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse Adiponectin Standard - Reconstitute the mouse Adiponectin Standard with 5.0 mL of Calibrator Diluent RD5-26 (1X). Do not substitute other diluents. This reconstitution produces a stock solution of 10 ng/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-26 (1X) into each tube. Use the standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted mouse Adiponectin Standard serves as the high standard (10 ng/mL). Calibrator Diluent RD5-26 (1X) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and control be assayed in duplicate.

1. Prepare reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of Standard, control, or sample* per well. Tap plate gently for one minute. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse Adiponectin Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Serum and plasma samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

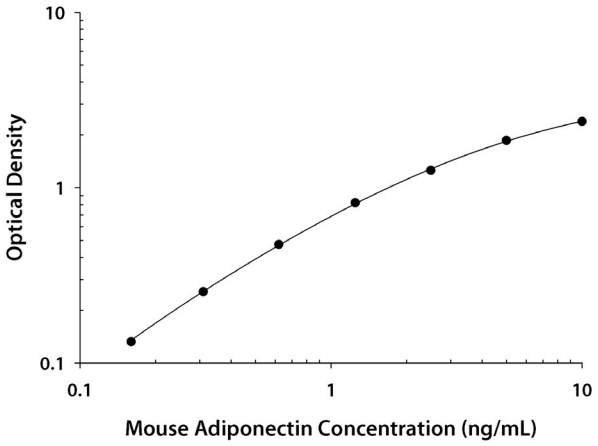
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create 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. The data may be linearized by plotting the log of the mouse 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 prior to assay, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.010 0.011	0.010	—
0.16	0.138 0.147	0.142	0.132
0.31	0.259 0.271	0.265	0.255
0.62	0.469 0.498	0.484	0.474
1.25	0.808 0.850	0.829	0.819
2.5	1.219 1.311	1.265	1.255
5	1.856 1.878	1.867	1.857
10	2.386 2.403	2.394	2.384

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty-three separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	23	23	23
Mean (ng/mL)	0.33	1.23	3.83	0.35	1.22	4.02
Standard deviation	0.022	0.072	0.221	0.021	0.061	0.258
CV (%)	6.7	5.9	5.8	6.0	5.0	6.4

RECOVERY

The recovery of mouse Adiponectin spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=7)	103	82-115%
Serum* (n=6)	98	85-114%
EDTA plasma* (n=4)	92	81-106%
Heparin plasma* (n=4)	93	77-110%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Fourteen assays were evaluated and the minimum detectable dose (MDD) of mouse Adiponectin ranged from 0.001-0.007 ng/mL. The mean MDD was 0.003 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse Adiponectin produced at R&D Systems. The recombinant mouse Adiponectin preparation contains a mixture of the trimeric, hexameric, and higher order multimeric full-length Adiponectin isoforms.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse Adiponectin in each matrix were diluted with Calibrator Diluent and then assayed.

		Cell culture supernates (n=4)	Tissue homogenates* (n=2)	Serum* (n=6)	EDTA plasma* (n=3)	Heparin plasma* (n=3)
1:2	Average % of Expected	93	97	97	95	95
	Range (%)	88-105	90-103	92-102	95-95	93-98
1:4	Average % of Expected	94	104	100	97	98
	Range (%)	88-105	98-109	94-103	93-100	97-99
1:8	Average % of Expected	95	108	101	96	96
	Range (%)	87-108	99-116	99-104	93-99	94-97
1:16	Average % of Expected	114	109	101	97	96
	Range (%)	114-114	101-117	97-107	93-101	93-100

*Samples were diluted prior to assay.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of mouse Adiponectin in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	7476	2652-15,528	2959
EDTA plasma (n=10)	6675	4408-8880	1423
Heparin plasma (n=10)	7198	5228-9702	1494

Cell Culture Supernates - Two lungs (1-2 mm pieces in 40 mL of medium) were cultured for 7 days in RPMI supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed for evaluation, assayed for levels of mouse Adiponectin, and measured 1.4 ng/mL.

Tissue Homogenates - Homogenates from spleen, liver, and fat tissue were assayed for mouse Adiponectin and measured 13 ng/mL, 30 ng/mL, and 51 ng/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant full-length mouse Adiponectin.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-26 (1X) and assayed for cross-reactivity. Preparations of the following factors at the same concentrations in a mid-range mouse Adiponectin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

CD27 Ligand
CD30 Ligand
CD40 Ligand
Fas Ligand
LT-α1/β2
LT-α2/β1
OX40 Ligand
RANK Ligand
TRAIL
TNF-α
TNF-α (truncated)
TWEAK

Recombinant human:

Adiponectin
C1qR/Fc Chimera

This assay does not recognize mouse gAdiponectin.

This assay does not detect recombinant rat Adiponectin (up to 2 µg/mL) or rat serum samples.

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