Adipoq (Rat) ELISA Kit

Catalog Number KA1026

96 assays

Version: 09

Intended for research use only

Table of Contents

Introduction

Background

Adiponectin, also known as adipocyte complement-related 30 kDa protein (ACRP30), is a secreted serum protein expressed exclusively in differentiated adipocytes. Studies indicated that decreased plasma adiponectin concentration is associated with insulin resistance (1), essential hypertension (2), and inflammation (3).

Principle of the Assay

The Adipoq (Rat) ELISA Kit is designed for detection of adiponectin in rat plasma, serum, urine, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures rat adiponectin in approximately 3 hours. A polyclonal antibody specific for rat adiponectin has been pre-coated onto a 96-well microplate with removable strips. Adiponectin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for rat adiponectin, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Rat Adiponectin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat adiponectin.	96 (8x12) wells
Sealing Tapes: Pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Rat Adiponectin Standard: Rat adiponectin in a buffered protein base (lyophilized).	250 ng
Biotinylated Rat Adiponectin Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against rat adiponectin.	60 µL
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
SP Conjugate (100x): A 100-fold concentrate.	80 µL
Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction.	12 mL

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ This product is for Research Use Only and is not intended for use in diagnostic procedures.
- ✓ Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- ✓ The Stop Solution is an acidic solution.
- ✓ The kit should not be used beyond the expiration date.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.
- ✓ Rat Adiponectin Standard: Reconstitute the Rat Adiponectin Standard (250 ng) with 2.5 mL of MIX Diluent to generate a 100 ng/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (100 ng/mL) 2-fold with equal volume of MIX Diluent to produce 50, 25, 12.5, 6.25, 3.125, and 1.563 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be stored at -20°C and used within 30 days.

Standard Point	Dilution	[Rat Acrp30] (ng/mL)
P1	1 part standard (100 ng/mL)	100.0
P2	1 part P1 + 1 part MIX Diluent	50.00
P3	1 part P2 + 1 part MIX Diluent	25.00
P4	1 part P3 + 1 part MIX Diluent	12.50
P5	1 part P4 + 1 part MIX Diluent	6.250
P6	1 part P5 + 1 part MIX Diluent	3.125
P7	1 part P6 + 1 part MIX Diluent	1.563
P8	MIX Diluent	0.000

- ✓ Biotinylated Rat Adiponectin Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 100-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be stored at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Sample Preparation

- ✓ Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 400-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The

undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant)

- ✓ Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 400-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes at 4°C to remove debris and collect supernatants. If necessary, dilute samples into MIX Diluent. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

- ✓ Refer to Dilution Guidelines below for further instruction.

Guidelines for Dilutions of 100-fold or Greater (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A. 4 µL sample: 396 µL buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 µL.	A. 4 µL sample: 396 µL buffer (100x) B. 4 µL of A : 396 µL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 µL.
1000x	100000x
A. 4 µL sample: 396 µL buffer (100x) B. 24 µL of A : 216 µL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 µL.	A. 4 µL sample: 396 µL buffer (100x) B. 4 µL of A : 396 µL buffer (100x) C. 24 µL of B : 216 µL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 µL.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Rat Adiponectin Standard or sample to each well. Gently tap plate to thoroughly coat the

wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour. Start the timer after the last addition.

4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents; hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of Biotinylated Rat Adiponectin Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 10 minutes or until the optimal blue color density develops.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

✓ Summary

1. Add 50 µL of Standard or Sample per well. Incubate for 1 hour.
2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour.
3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 10 minutes.
5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

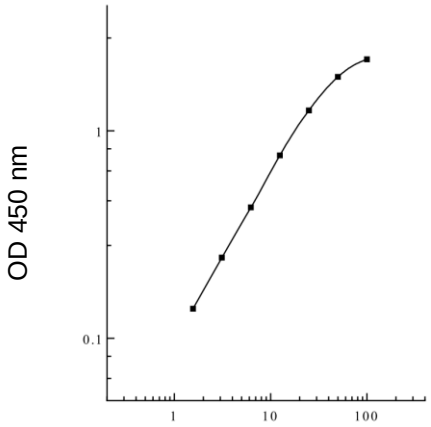
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

- ✓ Typical Data: The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/mL	OD	Average OD
P1	100.0	2.230 2.196	2.213
P2	50.00	1.832 1.804	1.818
P3	25.00	1.269 1.241	1.255
P4	12.50	0.770 0.754	0.762
P5	6.250	0.442 0.411	0.427
P6	3.125	0.250 0.239	0.245
P7	1.563	0.143 0.134	0.139
P8	0.000	0.036 0.033	0.035
Sample: Pooled Rat Sodium Citrate Plasma (400x)		0.812 0.765	0.789

- ✓ Standard Curve

  The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



[R. Acrp30] (ng/mL)

Performance Characteristics

- ✓ This assay recognizes both natural and recombinant rat adiponectin. It can detect both globular domain and full-length rat adiponectin.
- ✓ The minimum detectable dose of rat adiponectin as calculated by 2SD from the mean of a zero standard was established to be 1.1 ng/mL.
- ✓ Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- ✓ Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	2.7%	3.3%	5.2%	9.8%	9.9%	10.1%
Average CV (%)	3.7%			9.9%		

- ✓ Recovery

Standard Added Value	3.125 – 50 ng/mL
Recovery %	89-114%
Average Recovery %	98%

- ✓ Linearity

Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
200x	94%	91%
400x	101%	98%
800x	106%	105%

✓ Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	100%
Mouse	5%
Rat	100%
Swine	None
Rabbit	None
Human	100%

Resources

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of expired components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are dry after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Step performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.

Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

1. Tsao, T.S. et al. (2002) EJP 440:213-221
2. Adamczak, M. et al. AJH 16:72-75
3. Matsubara, M. et al. (2003) Eur J Endocrinol. 148(6): 657-62

Plate Layout

12								
11								
10								
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8								
7								
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5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H