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MOUSE ADIPONECTIN ENZYME IMMUNOASSAY KIT

catalogue # A05187

96 wells

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For research laboratory use only.
Not for diagnostic use.

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August 2012

MOUSE ADIPONECTIN EIA KIT

96 wells
Storage: 2-8℃
Expiry date: stated on the package

This kit contains:

- A covered 96 wells plate pre-coated with mouse anti-Adiponectin antibody, ready to use
- One vial of biotin labelled anti-mouse Adiponectin antibody, ready to use
- One vial of Streptavidin tracer, ready to use
- Two vials of mouse Adiponectin standard, lyophilised
- One vial of Substrate (TMB) solution, ready to use
- One vial of Stop solution (0.2 M H_2SO_4), ready to use
- Two vials of concentrated EIA Buffer (10x), liquid
- Two vials of Quality Controls: low and high, lyophilised
- One vial of concentrated Wash buffer (10x), liquid
- One instruction booklet
- One template sheet
- One well cover sheet

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 41 samples in duplicate.

PRECAUTIONS FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipet tip is used, aspirate a sample of reagent and dispense into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.
Not for diagnostic use.
Do not pipet liquids by mouth.
Do not use kit components beyond the expiration date.
Do not mix different lot numbers.
Do not eat, drink, or smoke in area in which kit reagents are handled.
Avoid splashing.

This kit contains components of animal origin.

Stop solution and Substrate solution are potential harmful solution. To avoid any contact, wear eye, hand, face and clothing protection when handling these reagents.

PRINCIPLE OF THE ASSAY

Adiponectin, also referred to as Acrp30, AdipoQ and GBP-28, is an 244 aminoacid protein, which is physiologically active, specifically and highly expressed in adipose cells (adipokine). Adiponectin forms homotrimers, which are the building blocks for higher order complexes found circulating in serum.

Paradoxically, adipose tissue-expressed adiponectin levels are inversely related to the degree of adiposity. A reduction in adiponectin serum levels is accompanied by insulin resistance states, such as obesity and type II diabetes mellitus. Adiponectin has been shown to increase insulin sensitivity and decrease plasma glucose by increasing tissue fat oxidation. It inhibits the inflammatory processes of atherosclerosis suppressing the expression of adhesion and cytokine molecules in vascular endothelial cells and macrophages, respectively.

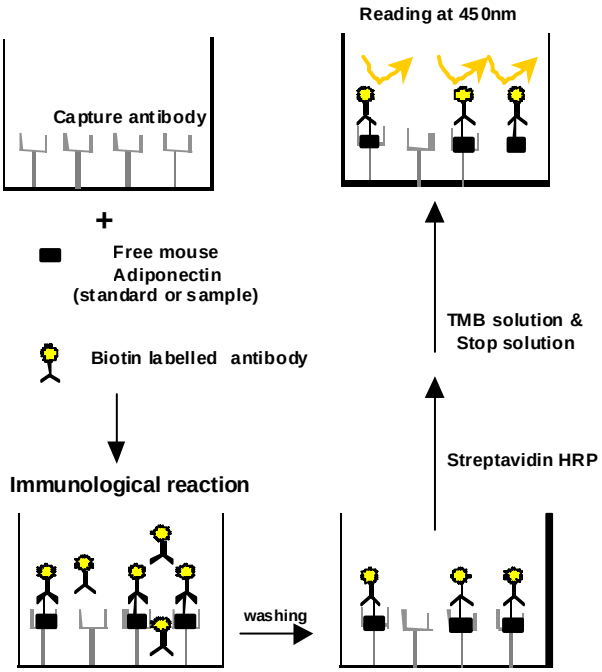
This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a monoclonal antibody specific of mouse adiponectin. This antibody will bind any mouse adiponectin introduced in the wells (sample or standard).
After one-hour incubation and a washing, biotin-labelled polyclonal anti-mouse Adiponectin antibody is added and incubated with captured Adiponectin during one hour.

After a thorough wash, streptavidin-horseradish peroxidase (HRP) tracer is added and incubated for 30 minutes.
This allows the two antibodies to form a sandwich by binding on different parts of the Adiponectin molecule.

The sandwich complex is immobilised on the plate so the excess reagents may be washed away. The concentration of the Adiponectin is then determined by measuring the enzymatic activity of the HRP using the hydrogen peroxide/TMB solution. The reaction is stopped by addition of sulphuric acid solution. The HRP tracer acts on TMB Reagent to form a yellow compound

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of the mouse adiponectin present in the well during the immunological incubation.

The principle of the assay is summarised below:



MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

FOR THE ASSAY

- Precision micropipettes (10 to 1000 µL)
- Spectrophotometer plate reader (450 nm +/- 10 nm filter)
- Microtitration washer (or washbottles)
- Microplate shaker
- Multichannel pipette 50-200 µL and disposable tips
- Distilled or deionised water
- Polypropylene tubes

SAMPLE PREPARATION

This assay may be used to measure mouse Adiponectin in mouse samples such as serum.

GENERAL PRECAUTIONS

☞All samples must be free of organic solvents prior to assay.

☞Samples should be assayed immediately after collection or should be stored frozen.

SERUM

Dilute samples 1/10 000 in EIA buffer preferably in two steps: add 10 µL sample to 990 µL EIA buffer (dilution 100x). Mix well. Add 10 µL of this dilution 100x to 990 µL EIA buffer for the final dilution. Mix well.
Serum samples should be stored frozen. It has been proved that the Adiponectin concentration in serum samples does not decrease after five thawing-freezing cycles. Nevertheless, repeated thawing-freezing should be avoided.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results!

REAGENT PREPARATION

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready to use, except the Standards, EIA buffer and the concentrated Wash buffer.

☞ EIA buffer
Dilute only the required amount of concentrated EIA buffer.
Otherwise dilute 22 mL of concentrated EIA buffer to 220 mL with distilled or deionised water.
Stability at 4℃: 1 week.

☞ Mouse Adiponectin standard
Reconstitute the vial with X mL of EIA buffer. The volume X is indicated on the vial of the corresponding standard. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. The concentration of the first standard is 8 ng/mL. Prepare 5 polypropylene tubes (for five other standards) and add 500 µL of EIA buffer into each tube. Add 500 µL of the first tube (containing the first standard) to the second tube. Continue this procedure to the other tubes. Thus, the standard concentrations are: 8 (S1), 4 (S2), 2 (S3), 1 (S4), 0.5 (S5), 0.25 ng/mL (S6), respectively.
Do not store the diluted standards.

☞ Quality Controls
Reconstitute the vials with X mL of EIA buffer. The volume X is indicated on the vial of the corresponding Quality Control. Allow them to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Do not store the reconstituted Quality Controls.

☞ Wash buffer
Dilute one vial of concentrated Wash buffer (100 mL, 10x) to 1000 mL with distilled or deionised water.
Stability at 4℃: 1 month

☞ Hydrogen peroxide/TMB solution
Substrate solution should remain colourless until added to the plate. Keep substrate solution protected from the light.

ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested on this page. The content of each well may be recorded on the sheet provided with the kit.

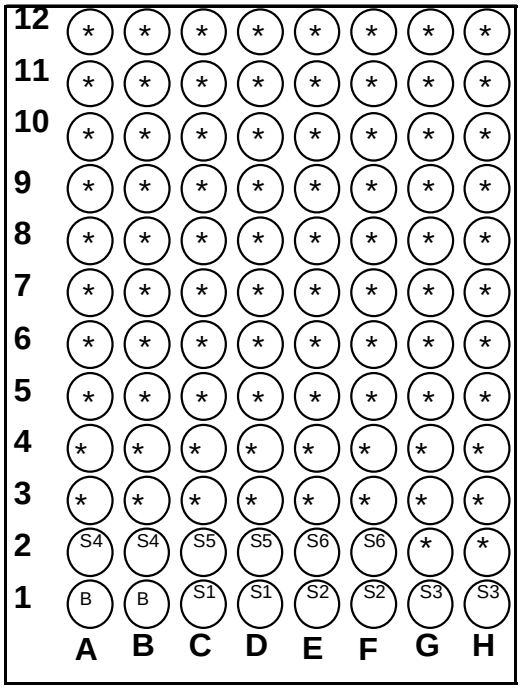
PIPETTING THE REAGENTS

All samples and reagents must reach room temperature prior performing the assay. Use different tips to pipet the buffer, standard, sample, tracer antiserum and other reagents.

- Mouse Adiponectin standard:
  Dispense 100 µL of the six standards (S1 to S6) in duplicate to appropriate wells. Start with the lowest concentration and equilibrate the tip in the next higher standard before pipetting.

- Quality Control and samples:
  Dispense in duplicate 100 µL of reconstituted Quality Control and samples to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

- EIA buffer
  Dispense in duplicate 100 µL of EIA buffer in Blank (B) wells.



B: Blank
S1-S6: Standards 1-6
*: Samples or Quality Controls

INCUBATING THE PLATE

- Incubate the plate for 1 hour at RT, shaking at 300 rpm on an orbital microplate shaker.
- Rinse the wells 3 times with the wash buffer (350 µL per well). After final wash, invert and tap the plate strongly against paper towel.
- Anti-adiponectin antibody Biotin Labelled: Dispense 100 µL to each well.
- Cover the plate with the cover sheet and incubate the plate for 1 hour at RT, shaking at 300 rpm on an orbital microplate shaker.
- Rinse the wells 3 times with the wash buffer (350 µL per well). After final wash, invert and tap the plate strongly against paper towel.
- Streptavidin-HRP tracer: Dispense 100 µL to each well.
- Cover the plate with the cover sheet and incubate the plate for 30 minutes at RT, shaking at 300 rpm on an orbital microplate shaker.
- Rinse the wells 3 times with the wash buffer (350 µL per well). After final wash, invert and tap the plate strongly against paper towel.

DEVELOPING AND READING THE PLATE

- Dispense 100 µL of Substrate solution to the 96 wells. Incubate in the dark during 10 minutes at room temperature (20-30°C). Avoid exposure to direct sun light. It is recommended to cover the plate with aluminium foil.
- Stop the colour development by adding 100 µL of Stop solution.
- Read the absorbance at 450 nm within 5 minutes following stop solution addition.

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the lowest standard (the highest absorbance of the calibration curve), perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine Adiponectin concentration of off-scale samples. ***The readings at 405 nm should not replace the on-scale readings at 450 nm.***

DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of TMB solution) from the absorbance readings of the rest of the plate. If not, do it now.

- Using a logit-log graph paper, plot the absorbance for each standard (y axis) versus concentration (x axis) of standards. Draw a best-fit line through the points.
- To determine the concentration of your samples, find the absorbance value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Most plate readers are supplied with curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

TYPICAL DATA

EXAMPLE DATA

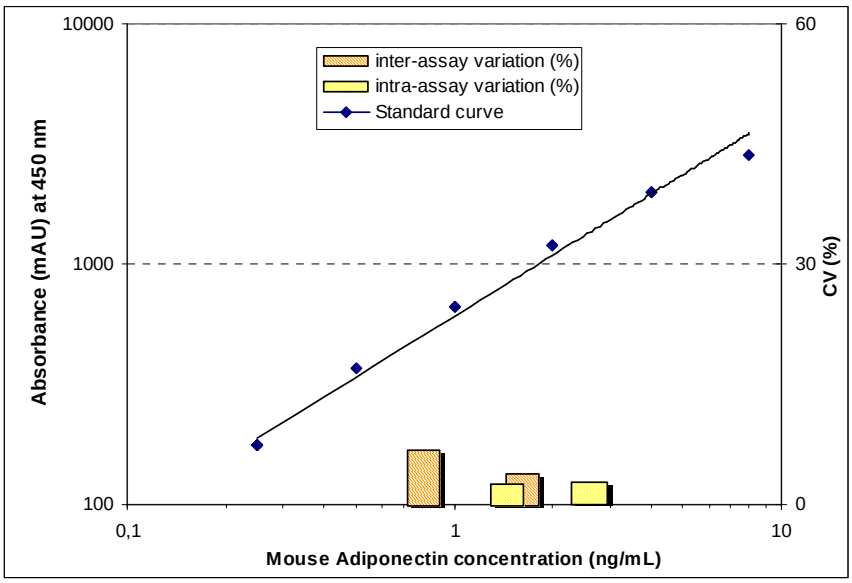
The following data are for demonstration purposes only. Your data may be different but still be correct. These data were obtained using all reagents as supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Mouse Adiponectin	mAU
Standard 8 ng/mL	2 849
Standard 4 ng/mL	1 971
Standard 2 ng/mL	1 189
Standard 1 ng/mL	660
Standard 0.5 ng/mL	369
Standard 0.25 ng/mL	177
QC High	1 873
QC Low	709

ACCEPTABLE RANGE

☞QC samples: see label on the vials.

MOUSE ADIPONECTIN STANDARD CURVE



ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of mouse Adiponectin has been validated for its use in serum.

☞Cross-reactivity:

Mouse leptin	<0.1 %
Mouse leptin receptor	<0.1 %
Mouse resistin	<0.1 %
Rat adiponectin	<0.3 %
Rabbit adiponectin	<0.1 %
Human adiponectin	<0.1 %
Cow adiponectin	<0.1 %
Horse adiponectin	<0.1 %
Hamster adiponectin	<0.1 %
Pig adiponectin:	<0.1 %

☞Sensitivity:

The limit of detection calculated as the concentration of mouse Adiponectin corresponding to the blank average minus three standard deviation is: 0.1 ng/mL.

☞Precision:

- Intra-assay (n=8)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	2.708	0.063	2.31
2	1.487	0.040	2.66
3	1.123	0.022	1.96
4	0.871	0.031	3.53

- Inter-assay (n=5)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	2.571	0.121	4.71
2	1.462	0.056	3.82
3	1.297	0.093	7.14
4	0.785	0.044	5.65

☞Recovery test:

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	0.90	-	-
	1.34	1.40	98.5
	1.82	1.90	97.2
	2.65	2.90	97.8
2	2.00	-	-
	2.52	2.50	101.0
	3.09	3.00	103.2
	3.94	4.00	98.6

☞Dilution test:

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	2.61	-	-
	1:2	1.15	1.24	92.6
	1:4	0.51	0.62	82.4
	1:8	0.26	0.31	83.4
2	-	5.26	-	-
	1:2	2.55	2.63	96.8
	1:4	1.22	1.32	92.9
	1:8	0.53	0.66	80.7

☞ Effect of freezing/thawing cycles:

No significant decline was observed in concentration of mouse Adiponectin in serum samples after repeated (5x) freezing/thawing cycle. Adiponectin concentration varies within the limit of 10%.

Number of serum samples	Number of freezing/thawing cycles	Mean %
N=13	0	100.0
	1	97.5
	3	99.5
	5	100.6

However, we recommend to avoid using a sample more than once frozen and thawed.

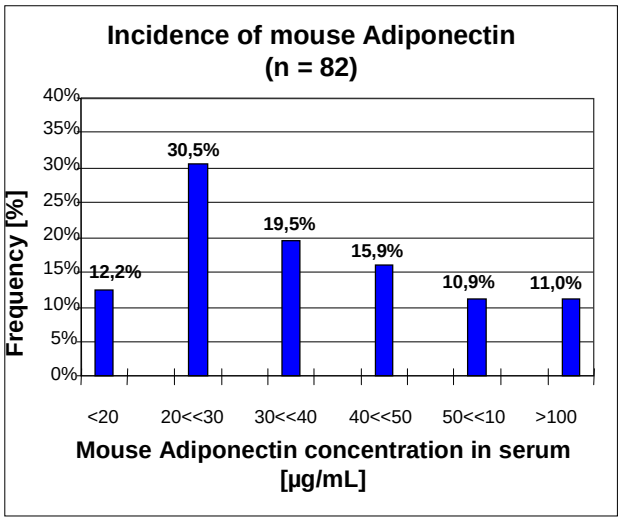
☞Stability of undiluted samples at 4℃:

No significant decline was observed in concentration of mouse adiponectin in serum samples after 14 days storage at 4℃. Adiponectin concentration vari es usually within the limit of 5%, rarely up to 15%. Nevertheless we recommend to store samples at –80℃ .

There is no difference between alternative of serum with and without preservatives.

☞ Normal values:

The following values were obtained when 82 sera from healthy BALB/c mice were assayed:



ASSAY TROUBLE SHOOTING

☞Absorbance values too low:

- One reagent has not been dispensed
- Incorrect preparation or reagent storage
- Assay performed before reagents reach room temperature

☞High signal and background in all wells:

- Inefficient washing
- Overdeveloping; incubation time should be reduced before adding Stop Solution

☞High dispersion of duplicates:

- Poor pipetting technique or irregular plate washing.

These are a few examples of problems that may occur. If you need further assistance, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (sales@spibio.com), and be sure to indicate the lot number of the kit (see outside of the box).

BIBLIOGRAPHY

- **Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF**. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem. 1995, 270, 26746-26749.*
- **Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K.** cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (adipose most abundant gene transcript 1). *Biochem Biophys Res Commun. 1996, 221, 286-289.*
- **Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y.** Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun. 1999, 257, 79-83.*
- **Saito K, Tobe T, Minoshima S, Asakawa S, Sumiya J, Yoda M, Nakano Y, Shimizu N, Tomita M.** Organization of the gene for gelatin-binding protein (GBP28). *Gene. 1999, 229, 67-73.*
- **Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y.** Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood. 2000, 96, 1723-1732.*
- **Das K, Lin Y, Widen E, Zhang Y, Scherer PE.** Chromosomal localization, expression pattern, and promoter analysis of the mouse gene encoding adipocyte-specific secretory protein Acrp30. *Biochem Biophys Res Commun. 2001, 280, 1120-1129.*
- **Tsao TS, Lodish HF, Fruebis J.** ACRP30, a new hormone controlling fat and glucose metabolism. *Eur J Pharmacol. 2002, 440, 213-21.*
- **Wang Y, Xu A, Knight C, Xu LY, Cooper GJ.** Hydroxylation and glycosylation of the four conserved lysine residues in the collagenous domain of adiponectin. Potential role in the modulation of its insulin-sensitizing activity. *J Biol Chem. 2002, 277, 19521-19529.*
- **Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, Ouchi N, Kihara S, Kawamoto T, Sumitsuji S, Funahashi T, Matsuzawa Y.** Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. *Diabetes. 2002, 51, 2325-2328.*
- **Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF.** Adiponectin and protection against type 2 diabetes mellitus. *Lancet. 2003, 361, 226-228.*
- **Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE.** Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. *J Biol Chem. 2003, 278, 9073-9085.*
- **Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, Ohnuma H, Igarashi M, Tominaga M, Kato T.** Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese Population: the Funagata study. *Diabetes Care. 2003 Jul;26(7):2015-20.*
- **Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, Uchida S, Ito Y, Takakuwa K, Matsui J, Takata M, Eto K, Terauchi Y, Komeda K, Tsunoda M, Murakami K, Ohnishi Y, Naitoh T, Yamamura K, Ueyama Y, Froguel P, Kimura S, Nagai R, Kadowaki T.** Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem. 2003, 278, 2461-2468.*

- **Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE.** Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. *J Biol Chem. 2003, 278, 9073-9085.*
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