DRG® Human Adiponectin ELISA (EIA-4574)

CE

RUO in the USA

Revised 12 Nov. 2010 RM (Vers. 6.1)

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The Human Adiponectin ELISA is a competitive enzyme immunoassay for measurement of human adiponectin.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

Features

- The total assay time is less than 3 hours.
- The kit measures total adiponectin in serum and plasma (EDTA, citrate, heparin).
- Assay format is 96 wells.
- Quality Controls are human serum based.
- Standards are recombinant adiponectin based.
- Components of the kit are provided ready to use or concentrated.

2 STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3 TEST PRINCIPLE

In the Human Adiponectin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with recombinant human adiponectin together with polyclonal anti-human adiponectin antibody conjugated to horseradish peroxidase (HRP). After a washing step, the HRP conjugate bound to the adiponectin immobilized on the wells is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured.

The absorbance is inversely proportional to the adiponectin concentration. A standard curve is constructed by plotting absorbance values against adiponectin of standards, and concentrations of unknown samples are determined using this standard curve.

4 PRECAUTIONS

- **For professional use only.**
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
- This kit contains components of animal origin. These materials should be handled as potentially infectious

- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.

5 TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed.
- Use thoroughly clean glassware.
- Use deionized (distilled) water, stored in clean containers.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

6 REAGENT SUPPLIED

Kit Components	State	Quantity
Antigen **Coated Microtiter Strips**	ready to use	96 wells
Conjugate Solution	ready to use	7 ml
Set of **Standards**	concentrated	7 x 0.22 ml
Quality **Control High**	ready to use	0.4 ml
Quality **Control Low**	ready to use	0.4 ml
Dilution Buffer	ready to use	2 x 13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	2 x 13 ml
Stop Solution	ready to use	9 ml
Product Data Sheet + Certificate of Analysis		1 pc

7 MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 50-200 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter preferably with reference wavelength 630 nm (alternatively another one from the interval 550 - 650 nm)
- Software package facilitating data generation and analysis (optional)

8 PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

Assay reagents supplied ready to use:

Antigen Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Conjugate Solution
Dilution Buffer
Substrate Solution
Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

Quality Controls High, Low

Refer to the Certificate of Analysis for current Quality Control concentration!!!

Quality Controls are ready to use, do not dilute them. (Quality Controls are supplied diluted 30x).

Stability and storage

Opened Quality controls are stable 3 months when stored at 2-8°C.

Assay reagents supplied concentrated:

Human Adiponectin Standards

Dilute each concentration of Standards 3x with the Dilution Buffer just prior to the assay,
e.g. 50 µl of Standard + 100 µl of Dilution Buffer for duplicates. Mix well (not to foam).

Stability and storage:

Opened standards are stable 3 months when stored at 2-8°C

Do not store the diluted Standard solutions.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution.

Example: 100 ml of Wash Solution Concentrate (10x)+ 900 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

9 PREPARATION OF SAMPLES

The kit measures adiponectin in serum and plasma (EDTA, citrate, heparin)..

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 30x with Dilution Buffer just prior to the assay
e.g.10 µl of sample + 290 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

See Stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of adiponectin.

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

10 ASSAY PROCEDURE

1. Pipet **50 µl** of diluted Standards, samples, Quality Controls and Dilution Buffer (=Blank), preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
2. Add **50 µl** of Conjugate Solution into each well.
3. Incubate the plate at room temperature (ca. 25°C) for **2 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
4. Wash the wells **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
5. Add **200 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
6. Incubate the plate for **10-15 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
7. Stop the colour development by adding **50 µl** of Stop Solution.
8. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 7.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, 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 standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 10	QC High	Sample 7	Sample 15	Sample 23	Sample 31
B	Standard 5	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
C	Standard 2	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	Standard 1	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	Standard 0.5	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	Standard 0.2	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	Standard 0.1	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

11 CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of adiponectin µg/ml in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentration of samples and Quality Controls calculated from the standard curve must be multiplied by a dilution factor of 10, because as standards are diluted 3x and samples and Quality Controls are diluted 30x, e.g. 1.05 µg/ml (from standard curve) x 10 (dilution factor) = 10.5 µg/ml.

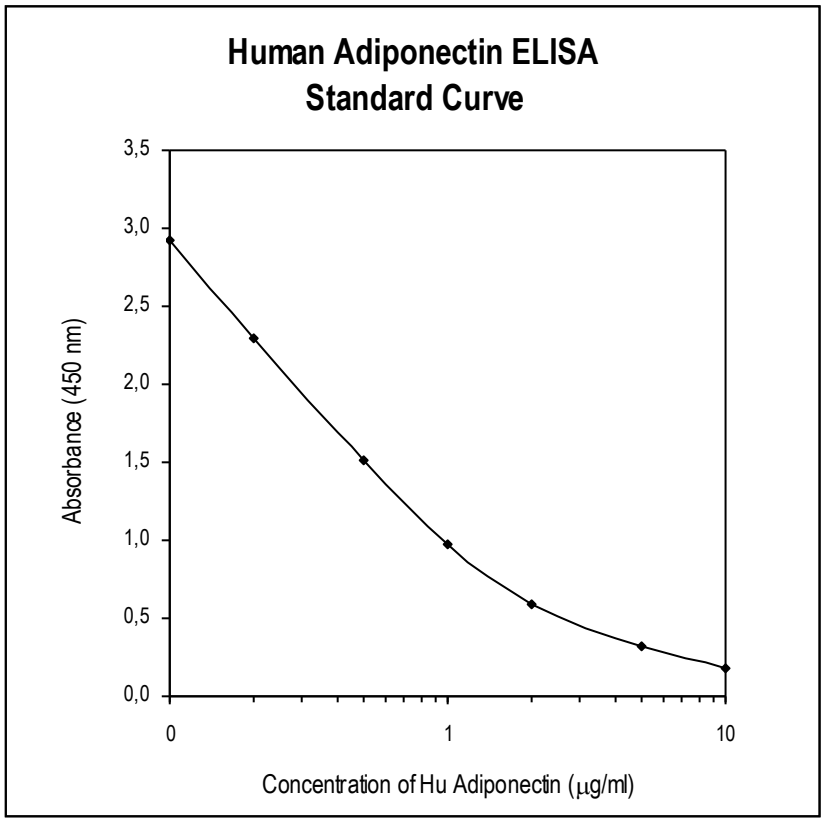


Figure 2: Typical Standard Curve for Human Adiponectin ELISA.

12 STABILITY OF SAMPLES STORED AT 2-8°C

Samples should be stored at -20°C. However, no significant decline in concentration of human adiponectin was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp, Period	Serum *(µg/ml)*	Plasma *(µg/ml)*		
			EDTA	Citrate	Heparin
1	-20°C	2.01	2.08	1.79	1.16
	2-8°C, 1 day	2.07	1.89	1.69	1.85
	2-8°C, 7 days	1.86	1.89	1.64	1.67
2	-20°C	7.30	6.76	6.56	5.78
	2-8°C, 1 day	7.24	6.83	6.39	6.20
	2-8°C, 7 days	7.10	7.07	5.87	6.20
3	-20°C	10.72	15.13	11.75	11.02
	2-8°C, 1 day	10.99	13.65	12.36	10.89
	2-8°C, 7 days	12.16	13.38	10.59	10.48

12.1 Effect of Freezing/Thawing

No significant decline was observed in concentration of human adiponectin in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum *(µg/ml)*	Plasma *(µg/ml)*		
			EDTA	Citrate	Heparin
1	1x	7.17	7.88	6.25	7.47
	3x	7.38	8.99	7.88	8.98
	5x	6.87	10.31	7.98	10.57
2	1x	10.86	13.16	10.83	10.60
	3x	13.53	14.47	13.21	11.51
	5x	11.23	11.22	8.64	10.96
3	1x	10.66	8.80	8.66	9.17
	3x	9.52	10.34	9.09	8.75
	5x	10.13	8.54	9.26	8.89

13 DEFINITION OF THE STANDARD

The recombinant human adiponectin is used as the Standard. The recombinant human adiponectin is produced in HEK293 cell line and contains 225 amino acid residues of the human adiponectin and 8 extra AA.

14 TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

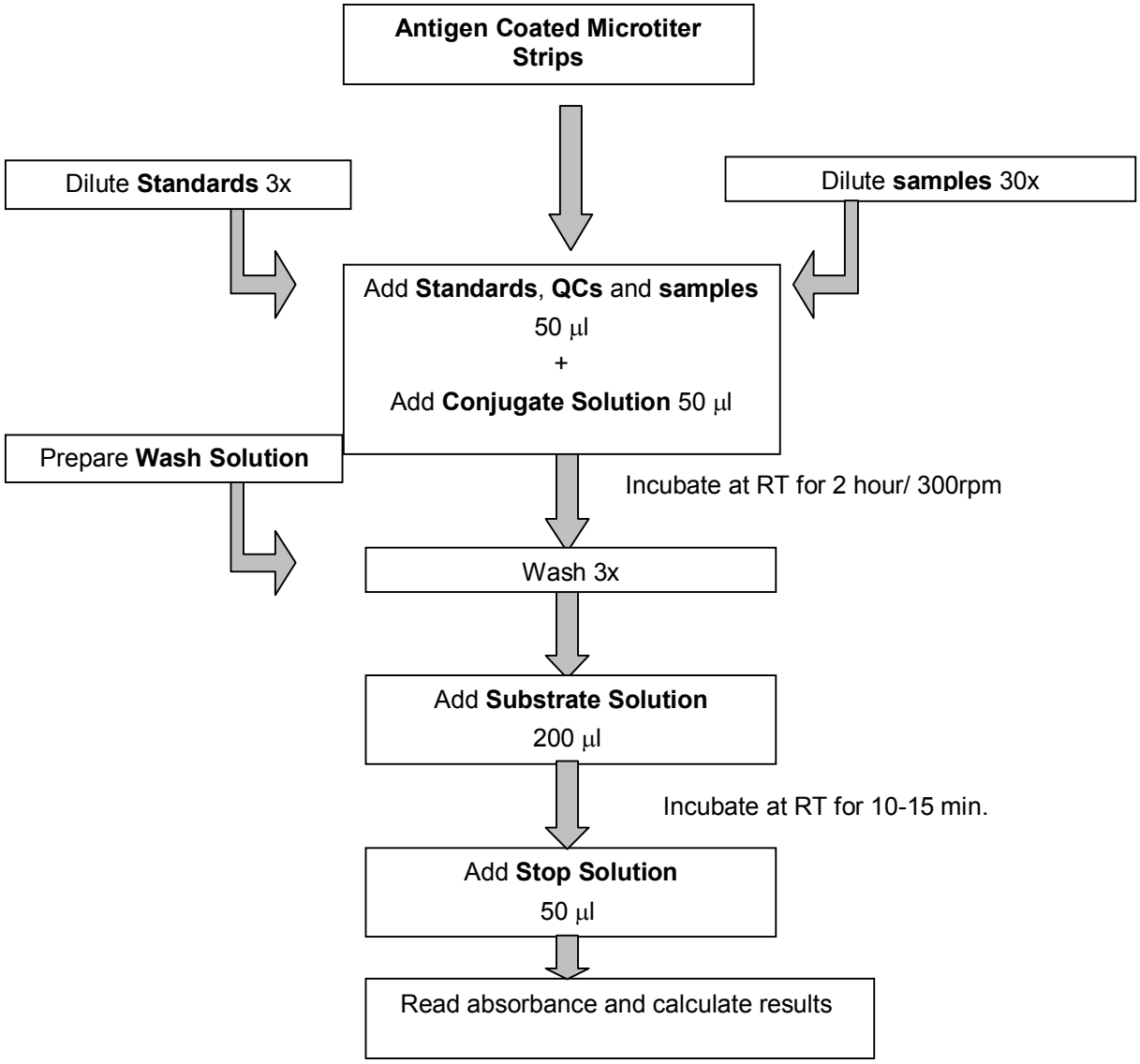
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

CE

RUO in the USA

Revised 12 Nov. 2010 RM (Vers. 6.1)

Assay Procedure Summary



15 REFERENCES

- Zhu W, Cheng KK, Vanhoutte PM, Lam KS, Xu A. Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention. Clin Sci (Lond). 2008 Mar;114(5):361-74.
- Takemura Y, Walsh K, Ouchi N. Adiponectin and cardiovascular inflammatory responses. Curr Atheroscler Rep. 2007 Sep;9(3):238-43.
- Kadowaki T, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. FEBS Lett. 2008 Jan 9;582(1):74-80. Epub 2007 Dec 3.
- Wang ZV, Scherer PE. Adiponectin, cardiovascular function, and hypertension. Hypertension. 2008 Jan;51(1):8-14. Epub 2007 Nov 12.
- Behre CJ. Adiponectin, obesity and atherosclerosis. Scand J Clin Lab Invest. 2007;67(5):449-58.
- Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin--a key adipokine in the metabolic syndrome. Diabetes Obes Metab. 2006 May;8(3):264-80.
- Berner HS, Lyngstadaas SP, Spahr A, Monjo M, Thommesen L, Drevon CaA, Syversen U., Reseland J.E.: Adiponectin and its receptors are expressed in bone-forming cells. Bone 2004; 35: 842-849
- Takahashi T, Zhu SJ, Sumino H, Saegusa S, Nakahashi T, Iwai K, Morimoto S, Kanda T: Inhibition of cyclooxygenase-2 enhances myocardial damage in a mouse model of viral myocarditis. Life Sci 2005
- Blüher M, Fasshauer M, Kralisch S, Schön MR, Krohn K, Paschke R: Regulation of adiponectin receptor R1 and R2 gene expression in adipocytes of C57BL/6 mice. Biochem Biophys Res Commun 2005; 329: 1127-1132
- Pilz S, Maerz W, Weihrauch G, Sargsyan K, Almer G, Nauck M, Boehm BO, Winkelmann BR, Mangge H: Adiponectin serum concentrations in men with coronary artery disease: The LUdwigshafen RIsk in Cardiovascular Health (LURIC) study. Clin Chim Acta 2005
- Haluzik M, Colombo C, Gavrilova O, Chua S, Wolf N, Chen M, Stannard B, Dietz KR, Le Roith D, Reitman ML. Genetic background (C57BL/6J versus FVB/N) strongly influences the severity of diabetes and insulin resistance in ob/ob mice. Endocrinology 2004; 145: 3258-3264
- Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, Funahashi T, Walsh K. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. J Biol Chem. 2004; 279: 1304-1309
- Ishikawa Y, Akasaka Y, Ishii T, Yoda-Murakami M, Choi-Miura NH, Tomita M, Ito K, Zhang L, Akishima Y, Ishihara M, Muramatsu M, Taniyama M. Changes in the distribution pattern of gelatin-binding protein of 28 kDa (adiponectin) in myocardial remodelling after ischaemic injury. Histopathology. 2003; 42: 43-52
- Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem. 2003; 278: 40352-40363
- Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 2003; 423: 762-769

- Combs TP, Pajvani UB, Berg AH, Lin Y, Jelicks LA, Laplante M, Nawrocki A, Rajala MW, Parlow AF, Cheeseboro L, Ding Y, Russell RG, Lindemann D, Hartley A, Baker GR, Obici S, Deshaies Y, Ludgate ME, Rossetti L, Scherer PE. A transgenic mouse with deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. Endocrinology 2003
- 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
- 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
- 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
- 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
- Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med. 2001; 7: 947-953
- 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, Gavrila 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 lipoathropy and obesity. Nat Med. 2001; 7: 941-946
- Fruebis J, Tsao TS, Javorschi S, Ebbets - Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. Proteolytic cleavage product of 30-kDa adiopcyte complement–related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA. 2001; 98: 2005-2010
- 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
- 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
- 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
- Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev. 2005 May;26(3):439-51.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

- Marinoni E, Letizia C, Ciardo F, Corona G, Moscarini M, Di Iorio R. Effects of prenatal betamethasone administration on leptin and adiponectin concentrations in maternal and fetal circulation. Am J Obstet Gynecol. 2008 Aug;199 (2):141.e1-6
- Serelis J, Kontogianni MD, Katsiougiannis S, Bletsa M, Tektonidou MG, Skopouli FN. Effect of anti-TNF treatment on body composition and serum adiponectin levels of women with rheumatoid arthritis. Clin Rheumatol. 2008 Jun;27 (6):795-7
- Catalan V, Gomez-Ambrosi J, Pastor C, Rotellar F, Silva C, Rodriguez A, Gil MJ, Cienfuegos JA, Salvador J, Vendrell J, Fruhbeck G. Influence of morbid obesity and insulin resistance on gene expression levels of AQP7 in visceral adipose tissue and AQP9 in liver. Obes Surg. 2008 Jun;18 (6):695-701
- Polak J, Kovacova Z, Holst C, Verdich C, Astrup A, Blaak E, Patel K, Oppert JM, Langin D, Martinez JA, Sorensen TI, Stich V. Total adiponectin and adiponectin multimeric complexes in relation to weight loss-induced improvements in insulin sensitivity in obese women: the NUGENOB study. Eur J Endocrinol. 2008 Apr;158 (4):533-41
- Vaverkova H, Karasek D, Novotny D, Jackuliakova D, Halenka M, Lukes J, Frohlich J. Positive association of adiponectin with soluble vascular cell adhesion molecule sVCAM-1 levels in patients with vascular disease or dyslipidemia. Atherosclerosis. 2008 Apr;197 (2):725-31
- Golledge J, Jayalath R, Oliver L, Parr A, Schurgers L, Clancy P. Relationship between CT anthropometric measurements, adipokines and abdominal aortic calcification. Atherosclerosis. 2008 Mar;197 (1):428-34
- Benedict C, Kern W, Schultes B, Born J, Hallschmid M. Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. J Clin Endocrinol Metab. 2008 Apr;93 (4):1339-44
- Chalvatzas N, Dafopoulos K, Kosmas G, Kallitsaris A, Pournaras S, Messinis IE. Effect of ovarian hormones on serum adiponectin and resistin concentrations. Fertil Steril. 2008 Mar 4;
- Schnabel R, Messow CM, Lubos E, Espinola-Klein C, Rupprecht HJ, Bickel C, Sinning C, Tzikas S, Keller T, Genth-Zotz S, Lackner KJ, Munzel TF, Blankenberg S. Association of adiponectin with adverse outcome in coronary artery disease patients: results from the AtheroGene study. Eur Heart J. 2008 Mar;29 (5):649-57
- Catalan V, Gomez-Ambrosi J, Rodriguez A, Silva C, Rotellar F, Gil MJ, Cienfuegos JA, Salvador J, Fruhbeck G. Expression of caveolin-1 in human adipose tissue is upregulated in obesity and obesity-associated type 2 diabetes mellitus and related to inflammation. Clin Endocrinol (Oxf). 2008 Feb;68 (2):213-9
- Lamounier-Zepter V, Bornstein SR, Kunes J, Zicha J, Krsek M, Ehrhart-Bornstein M, Ziegler CG, Kiessling A, Funk RH, Haluzik M. Adrenocortical changes and arterial hypertension in lipoatrophic A-ZIP/F-1 mice. Mol Cell Endocrinol. 2008 Jan 2;280 (1-2):39-46
- Avignon A, Sultan A, Piot C, Mariano-Goulart D, Thuan Dit Dieudonne JF, Cristol JP, Dupuy AM. Osteoprotegerin: a novel independent marker for silent myocardial ischemia in asymptomatic diabetic patients. Diabetes Care. 2007 Nov;30 (11):2934-9
- Catalan V, Gomez-Ambrosi J, Ramirez B, Rotellar F, Pastor C, Silva C, Rodriguez A, Gil MJ, Cienfuegos JA, Fruhbeck G. Proinflammatory cytokines in obesity: impact of type 2 diabetes mellitus and gastric bypass. Obes Surg. 2007 Nov;17 (11):1464-74
- Cabre A, Lazaro I, Girona J, Manzanares J, Marimon F, Plana N, Heras M, Masana L. Retinol-binding protein 4 as a plasma biomarker of renal dysfunction and cardiovascular disease in type 2 diabetes. J Intern Med. 2007 Oct;262 (4):496-503
- Gomez-Ambrosi J, Catalan V, Ramirez B, Rodriguez A, Colina I, Silva C, Rotellar F, Mugueta C, Gil MJ, Cienfuegos JA, Salvador J, Fruhbeck G . Plasma osteopontin levels and expression in adipose tissue are increased in obesity. J Clin Endocrinol Metab . Sep;92(9):3719-27 (2007)

- Westphal S, Borucki K, Taneva E, Makarova R, Luley C. Extended-release niacin raises adiponectin and leptin. Atherosclerosis. 2007 Aug;193 (2):361-5
- Saely CH, Risch L, Hoefle G, Rein P, Muendlein A, Marte T, Aczel S, Langer P, Drexel H . Low serum adiponectin is independently associated with both the metabolic syndrome and angiographically determined coronary atherosclerosis. Clin Chim Acta . Aug;383(1-2):97-102 (2007)
- Vitkova M, Klimcakova E, Kovacikova M, Valle C, Moro C, Polak J, Hanacek J, Capel F, Viguerie N, Richterova B, Bajzova M, Hejnova J, Stich V, Langin D . Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity. J Clin Endocrinol Metab . Jun;92(6):2330-5 (2007)
- Otto C, Otto B, Frost RJ, Vogeser M, Pfeiffer AF, Spranger J, Parhofer KG. Short-term therapy with atorvastatin or fenofibrate does not affect plasma ghrelin, resistin or adiponectin levels in type 2 diabetic patients with mixed hyperlipoproteinaemia. Acta Diabetol. 2007 Jun;44 (2):65-8
- Moro C, Klimcakova E, Lolmede K, Berlan M, Lafontan M, Stich V, Bouloumie A, Galitzky J, Arner P, Langin D . Atrial natriuretic peptide inhibits the production of adipokines and cytokines linked to inflammation and insulin resistance in human subcutaneous adipose tissue. Diabetologia . May;50(5):1038-47 (2007)
- Catalan V, Gomez-Ambrosi J, Rotellar F, Silva C, Gil MJ, Rodriguez A, Cienfuegos JA, Salvador J, Fruhbeck G . The obestatin receptor (GPR39) is expressed in human adipose tissue and is down-regulated in obesity-associated type 2 diabetes mellitus. Clin Endocrinol (Oxf) . Apr;66(4):598-601 (2007)
- Shin MJ, Kang SM, Jang Y, Lee JH, Oh J, Chung JH, Chung N. Serum retinol binding protein 4 levels are associated with serum adiponectin levels in non-diabetic, non-obese subjects with hypercholesterolemia. Clin Chim Acta. 2007 Mar;378 (1-2):227-9
- Risch L, Saely C, Hoefle G, Rein P, Langer P, Gouya G, Marte T, Aczel S, Drexel H. Relationship between glomerular filtration rate and the adipokines adiponectin, resistin and leptin in coronary patients with predominantly normal or mildly impaired renal function. Clin Chim Acta. 2007 Feb;376 (1-2):108-13
- Dieplinger B, Poelz W, Haltmayer M, Mueller T. Association of adiponectin and amino terminal proBNP in peripheral arterial disease. Clin Chim Acta. 2007 Feb;377 (1-2):192-7
- Mojiminiyi OA, Abdella NA, Al Arouj M, Ben Nakhi A. Adiponectin, insulin resistance and clinical expression of the metabolic syndrome in patients with Type 2 diabetes. Int J Obes (Lond). 2007 Feb;31 (2):213-20
- Delfini E, Petramala L, Caliumi C, Cotesta D, De Toma G, Cavallaro G, Panzironi G, Diacinti D, Minisola S, D' Erasmo E, Mazzuoli GF, Letizia C . Circulating leptin and adiponectin levels in patients with primary hyperparathyroidism. Metabolism . Jan;56(1):30-6 (2007)
- Bobbert T, Wegewitz U, Brechtel L, Freudenberg M, Mai K, Mohlig M, Diederich S, Ristow M, Rochlitz H, Pfeiffer AF, Spranger J. Adiponectin oligomers in human serum during acute and chronic exercise: relation to lipid metabolism and insulin sensitivity. Int J Sports Med. 2007 Jan;28 (1):1-8
- Risch L, Guenter H, Saely C, Berchthold S, Weber M, Gouya G, Rein P, Langer P, Marte T, Aczel S, Drexel H . Evaluation of two fully automated novel enzyme-linked immunosorbent assays for the determination of human adiponectin in serum. Clin Chim Acta . Nov;373(1-2):121-6 (2006)
- Pilz S, Mangge H, Wellnitz B, Seelhorst U, Winkelmann BR, Tiran B, Boehm BO, Marz W . Adiponectin and mortality in patients undergoing coronary angiography. J Clin Endocrinol Metab . Nov;91(11):4277-86 (2006)
- Bersinger NA, Birkhauser MH, Wunder DM . Adiponectin as a marker of success in intracytoplasmic sperm injection/embryo transfer cycles. Gynecol Endocrinol . Sep;22(9):479-83 (2006)

- Baranova A, Gowder SJ, Schlauch K, Elariny H, Collantes R, Afendy A, Ong JP, Goodman Z, Chandhoke V, Younossi ZM. Gene expression of leptin, resistin, and adiponectin in the white adipose tissue of obese patients with non-alcoholic fatty liver disease and insulin resistance. Obes Surg. 2006 Sep;16 (9):1118-25
- Senolt L, Pavelka K, Housa D, Haluzik M . Increased adiponectin is negatively linked to the local inflammatory process in patients with rheumatoid arthritis. Cytokine . Sep;35(5-6):247-52 (2006)
- Giri S, Rattan R, Hag E, Khan M, Yasmin R, Won JS, Key L, Singh AK, Singh I . AICAR inhibits adipocyte differentiation in 3T3L1 and restores metabolic alterations in diet-induced obesity mice model. Nutr Metab (Lond) . Aug 10;3:31 (2006)
- Shin MJ, Lee JH, Jang Y, Park E, Oh J, Chung JH, Chung N: . Insulin resistance, adipokines, and oxidative stress in nondiabetic, hypercholesterolemic patients: leptin as an 8-epi-prostaglandin F2alpha determinant. Metabolism . Jul;55(7):918-22 (2006)
- Stejskal D, Proskova J, Solichova P. Adiponectin added into the plasma of healthy probands does not affect platelet aggregability. Biomed Pap Med Fac Univ Palack. 2006 Jul;150 (1):89-90
- von Eynatten M, Hamann A, Twardella D, Nawroth PP, Brenner H, Rothenbacher D . Relationship of adiponectin with markers of systemic inflammation, atherogenic dyslipidemia, and heart failure in patients with coronary heart disease. Clin Chem . May;52(5):853-9 (2006)
- Weickert MO, Mohlig M, Schofl C, Arafat AM, Otto B, Viehoff H, Koebnick C, Kohl A, Spranger J, Pfeiffer AF . Cereal fiber improves whole-body insulin sensitivity in overweight and obese women. Diabetes Care . Apr;29(4):775-80 (2006)
- Otto C, Otto B, Goke B, Pfeiffer AF, Lehrke M, Vogeser M, Spranger J, Parhofer KG . Increase in adiponectin levels during pioglitazone therapy in relation to glucose control, insulin resistance as well as ghrelin and resistin levels. J Endocrinol Invest . Mar;29(3):231-6 (2006)
- Mohlig M, Freudenberg M, Bobbert T, Ristow M, Rochlitz H, Weickert MO, Pfeiffer AF, Spranger J . Acetylsalicylic acid improves lipid-induced insulin resistance in healthy men. J Clin Endocrinol Metab . Mar;91(3):964-7 (2006)
- Pilz S, Maerz W, Weihrauch G, Sargsyan K, Almer G, Nauck M, Boehm BO, Winkelmann BR, Mangge BR . Adiponectin serum concentrations in men with coronary artery disease: the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study. Clin Chim Acta . Feb;364(1-2):251-5 (2006)
- Karaduman M, Sengul A, Oktenli C, Pekel A, Yesilova Z, Musabak U, Sanisoglu SY, Gunay C, Baysan O, Kocar IH, Tatr H, Ozata M . Tissue levels of adiponectin, tumour necrosis factor-alpha, soluble intercellular adhesion molecule-1 and heart-type fatty acid-binding protein in human coronary atherosclerotic plaques. Clin Endocrinol (Oxf) . Feb;64(2):196-202 (2006)
- Spranger J, Verma S, Gohring I, Bobbert T, Seifert J, Sindler AL, Pfeiffer A, Hileman SM, Tschop M, Banks WA . Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes . Jan;55(1):141-7 (2006)
- Chu MC, Cosper P, Orio F, Carmina E, Lobo RA. Insulin resistance in postmenopausal women with metabolic syndrome and the measurements of adiponectin, leptin, resistin, and ghrelin. Am J Obstet Gynecol. 2006 Jan;194 (1):100-4
- Dieplinger B, Poelz W, Haltmayer M, Mueller T . Hypoadiponectinemia is associated with symptomatic atherosclerotic peripheral arterial disease. Clin Chem Lab Med . 44(7):830-3 (2006)
- Tsioufis C, Dimitriadis K, Chatzis D, Vasiliadou C, Tousoulis D, Papademetriou V, Toutouzas P, Stefanadis C, Kallikazaros I . Relation of microalbuminuria to adiponectin and augmented C-reactive protein levels in men with essential hypertension. Am J Cardiol . Oct 1;96(7):946-51 (2005)

- Bobbert A, Rochlitz H, Wegewitz U, Akpulat S, Mai K, Weickert MO, Mohlig M, Pfeiffer AF, Spranger J. Changes of adiponectin oligomer composition by moderate weight reduction. Diabetes . Sep;54(9):2712-9 (2005)
- Avignon A, Sultan A, Piot C, Elaerts S, Cristol JP, Dupuy AM . Osteoprotegerin is associated with silent coronary artery disease in high-risk but asymptomatic type 2 diabetic patients. Diabetes Care . Sep;28(9):2176-80 (2005)
- Pilz S, Horejsi R, Moller R, Almer G, Scharnagl H, Stojakovic T, Dimitrova R, Weihrauch G, Borkenstein M, Maerz W, Schauenstein K, Mangge H. Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. J Clin Endocrinol Metab. 2005 Aug;90 (8):4792-6
- Stejskal D, Ruzicka V, Fanfrdlova G, Kolar V, Bartek J . High adiponectin and TNF-alpha levels in moderate drinkers suffering from liver steatosis: comparison with non drinkers suffering from similar hepatopathy. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub . Jun;149(1):93-9 (2005)
- Bersinger NA, Smarason AK . Pre-eclampsia: increased, unchanged, and decreased serum markers in comparison to healthy third trimester pregnancy. A synopsis. Immuno-analyse et biologie spécialisée . 20: 353-359 (2005)
- Stejskal D, Ruzicka V, Adamovska S, Jurakova R, Proskova J, Jedelsky L, Bartek J . Adiponectin concentrations as a criterion of metabolic control in persons with type 2 diabetes mellitus?. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub . Dec;147(2):167-72 (2003)
- Stejskal D, Bartek J . Adiponectin in patients with various stages of coronary heart disease - comparison of its concentration in coronary arteries and peripheral venous circulation. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub . Dec;147(2):161-6 (2003)

16 PLATE LAYOUT

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H