Version 12-09/24

User's Manual

Adiponectin ELISA Kit

REF **DEIA-BJ2889**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Human Adiponectin ELISA is a competitive enzyme immunoassay for the quantitative measurement of human adiponectin. This kit has a strong cross reactivity with monkey serum samples with a 1:30 dilution.

General Description

Adiponectin, also referred to as Acrp30, AdipoQ and GBP-28, is a recently discovered 244 aminoacid protein, the product of the apM1 gene, which is physiologically active and specifically and highly expressed in adipose cells. The protein belongs to the soluble defence collagen superfamily; it has a collagen-like domain structurally homologous with collagen VIII and X and complement factor C1q-like globular domain. Adiponectin forms homotrimers, which are the building blocks for higher order complexes found circulating in serum. Together, these complexes make up approximately 0.01% of total serum protein. Adiponectin receptors AdipoR1 and AdipoR2 have been recently cloned; AdipoR1 is abundantly expressed in skeletal muscle,

whereas AdipoR2 is predominantly expressed in the liver. Paradoxically, adipose tissueexpressed adiponectin levels are inversely related to the degree of adiposity. Adiponectin concentrations correlate negatively with glucose, insulin, triglyceride concentrations, liver fat content and body mass index and positively with high-density lipoprotein-cholesterol levels, hepatic insulin sensitivity and insulin-stimulated glucose disposal. Adiponectin has been shown to increase insulin sensitivity and decrease plasma glucose by increasing tissue fat oxidation.

Clinical studies have shown that low adiponectin levels are associated with insulin resistance and precede the onset of type 2 diabetes. Diabetic patients have low levels of adiponectin and even lower levels of adiponectin were observed in patients with poorly controlled type 2 diabetes and in diabetic patients with coronary heart disease. Hypoadiponectinemia is also closely associated with the metabolic syndrome and with the hypertriglyceridemic waist. Non-alcoholic fatty liver disease is described as part of the metabolic syndrome and levels of adiponectin have inverse association with liver enzymes and fatty liver disease. The key finding is that low adiponectin serum levels predict type 2 diabetes independent of other risk factors.

Adiponectin also inhibits the inflammatory processes of atherosclerosis suppressing the expression of adhesion and cytokine molecules in vascular endothelial cells and macrophages, respectively. This adipokine plays a role as a scaffold of newly formed collagen in myocardial remodelling after ischaemic injury and also stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signalling in endothelial cells. Low serum adiponectin levels are found in patients with coronary artery disease.

Moreover, high circulating levels of adiponectin are associated with decreased risk of myocardial infarction, independent of other factors.

Altogether, monitoring of adiponectin levels and monitoring of processes that affect its production or its receptors are promising targets for prevention and treatment of obesity, insulin resistance, hyperlipidemia and atherosclerosis.

Clinical application and areas of investigation:

Energy metabolism and body weight regulation

Metabolic syndrome

Type 2 diabetes

Coronary artery disease

Atherosclerosis

Principles of Testing

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 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.

Reagents And Materials Provided

1. Antigen Coated Microtiter Strips, ready to use, 96 wells
2. Conjugate Solution, ready to use, 7 mL
3. Set of Standards concentrated, 7 × 0.22 mL
4. Quality Control HIGH, ready to use, 0.4 mL
5. Quality Control LOW, ready to use, 0.4 mL
6. Dilution Buffer, ready to use, 2 × 13 mL
7. Wash Solution Conc. (10×) concentrated, 100 mL
8. Substrate Solution, ready to use, 2 × 13 mL
9. Stop Solution, ready to use, 9 mL

Materials Required But Not Supplied

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

Storage

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

Specimen Collection And Preparation

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 30× 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.

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

Notes:

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

Centrifuge liquid containing microtube vials before opening.

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

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

1. Assay reagents supplied ready to use:

a. 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.

b. Conjugate Solution / Dilution Buffer / Substrate Solution / Stop Solution

Stability and storage: Opened reagents are stable 3 months when stored at 2-8°C.

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 30×).

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

Note:

Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with IFU and CoA and that ELISA test was carried out properly.

2. Assay reagents supplied concentrated:

a. Human Adiponectin Standards

Dilute each concentration of Standards 3× 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.

b. Wash Solution Conc. (10×)

Dilute Wash Solution Concentrate (10×) ten-fold in distilled water to prepare a 1× working solution. Example: 100 ml of Wash Solution Concentrate (10×) + 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 (10×) is stable 3 months when stored at 2-8°C.

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 hours, 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 1: 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.

Calculation

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 30×, e.g. 1.05 µg/ml (from standard curve) × 10 (dilution factor) = 10.5 µg/ml.

Typical Standard Curve

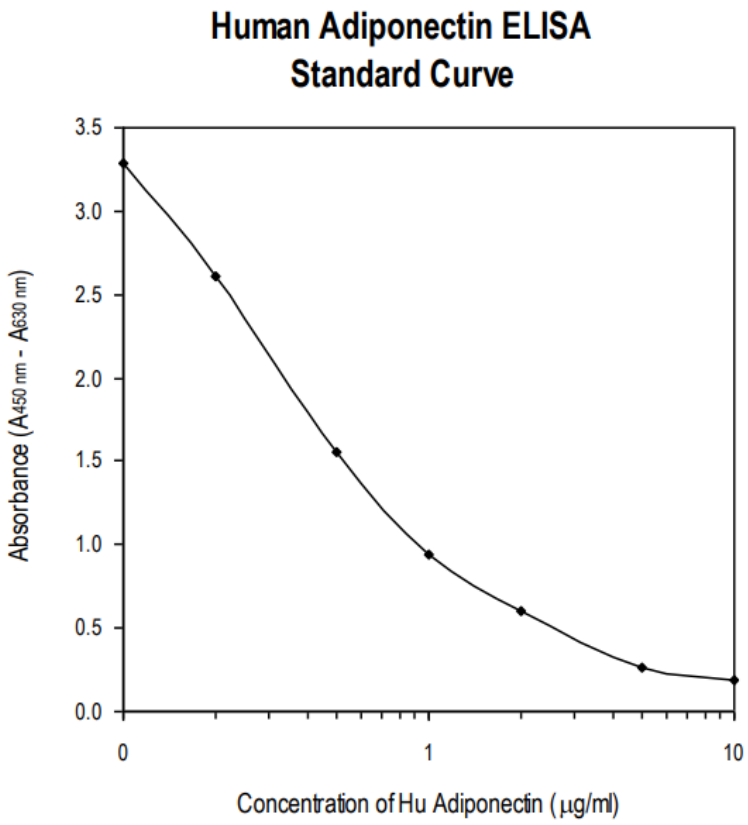

Figure 2: Typical Standard Curve for Human Adiponectin ELISA.

Performance Characteristics

Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

Volunteer No.	Serum (µg/ml)	Plasma (µg/ml)		
		EDTA	Citrate	Heparin
1	7.37	6.01	5.52	6.23
2	5.52	6.71	4.97	6.19
3	4.57	3.84	3.63	3.67
4	6.57	7.87	6.98	9.05
5	12.89	11.54	11.88	11.83
6	13.72	15.42	13.20	16.32
7	5.82	4.88	3.95	4.81
8	15.29	14.74	15.66	16.97
9	11.43	10.03	9.95	10.44
10	5.93	5.71	6.05	5.39
Mean (µg/ml)	**8.9**	**8.7**	**8.2**	**9.4**
Mean Plasma/Serum (%)	**-**	**97.4**	**91.8**	**105.6**
Coefficient of determination R^2	**-**	**0.92**	**0.96**	**0.91**

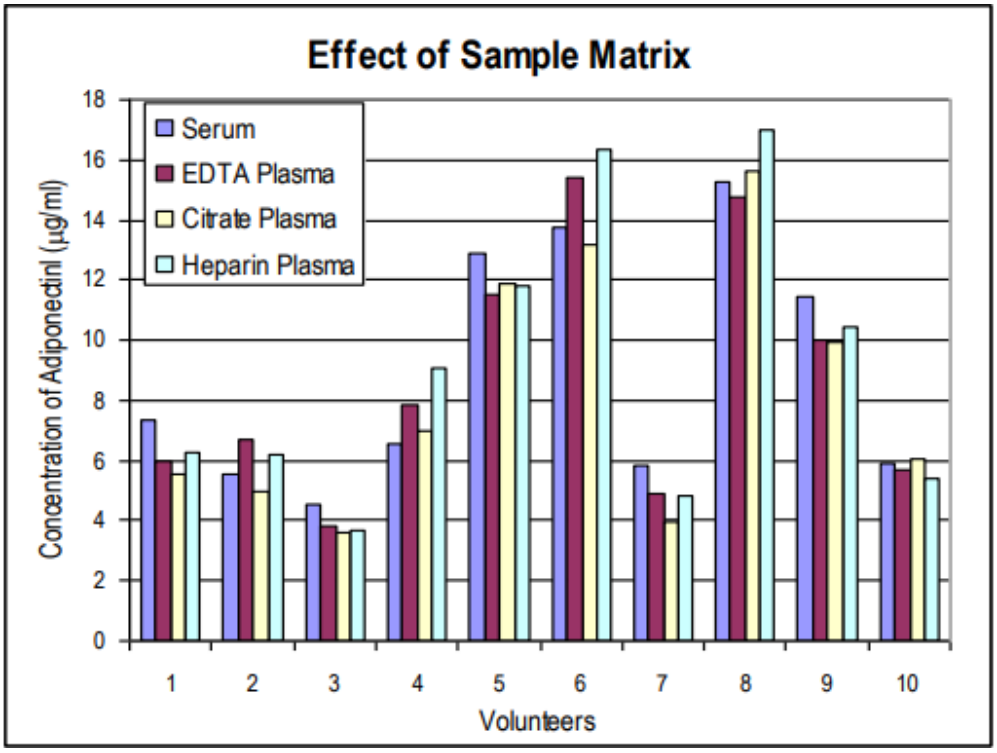

Figure 3: Adiponectin levels measured using Human Adiponectin ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (µg/ml)	SD (µg/ml)	CV (%)
1	11.71	0.69	5.9
2	12.28	0.481	3.9

Inter-assay (Run-to-Run) (n=8)

Sample	Mean (µg/ml)	SD (µg/ml)	CV (%)
1	8.23	0.52	6.3
2	19.86	1.39	7.0

Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance lower than mean absorbance of blank* minus three standard deviations of the absorbance of blank: A_{blank} - 3×SD_{blank}) is calculated from the real adiponectin values in wells and is 26 ng/ml.

***Dilution Buffer is pipetted into blank wells.**

Results exceeding adiponectin level of 100 µg/ml should be repeated with more diluted samples (e.g. 60×). Dilution factor needs to be taken into consideration in calculating the adiponectin concentration.

Specificity

The antibodies used in this ELISA are specific for human adiponectin. The assay recognizes natural and recombinant human adiponectin (full-length protein, mutationmodified trimer-only-forming protein, and globular domain). Determination of adiponectin does not interfere with hemoglobin (1.0 mg/ml), bilirubin (170 µmol/l) and triglycerides (5.0 mmol/l). Adiponectin was measured in some of adipose tissue extracts, however most of the extract adiponectin levels were bellow the assay detection limit. No crossreactivity has been observed for human leptin, leptin receptor and resistin at 100 ng/ml.

Sera of several mammalian species were measured in the assay. See results below.

Mammalian serum sample	Observed crossreactivity
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (μg/ml)	Expected (μg/ml)	Recovery O/E (%)
1	-	18.05	-	-
	2x	9.28	9.02	102.8
	4x	4.39	4.51	97.3
	8x	2.53	2.26	112.7
2	-	23.56	-	-
	2x	10.15	11.78	86.2
	4x	5.64	5.89	95.8
	8x	3.08	2.94	104.5

Recovery

Serum samples were spiked with different amounts of human adiponectin and assayed.

Sample	Observed (μg/ml)	Expected (μg/ml)	Recovery O/E (%)
1	5.10	-	-
	10.39	10.10	102.9
	15.57	15.10	103.1
	23.19	25.10	92.4
2	10.94	-	-
	16.18	15.94	101.5
	21.14	20.94	101.0
	30.02	30.94	100.3

Interferences

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

Effect of Freezing/Thawing

No significant decline was observed in concentration of human adiponectin in serum and plasma samples after repeated (5×) 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

Precautions

For professional use only

1. Wear gloves and laboratory coats when handling immunodiagnostic materials.
2. Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
3. 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.
4. 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.
5. The materials must not be pipetted by mouth.

TECHNICAL HINTS

1. Reagents with different lot numbers should not be mixed.
2. Use thoroughly clean glassware.
3. Use deionized (distilled) water, stored in clean containers.
4. Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
5. Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.
6. 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.
7. Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.