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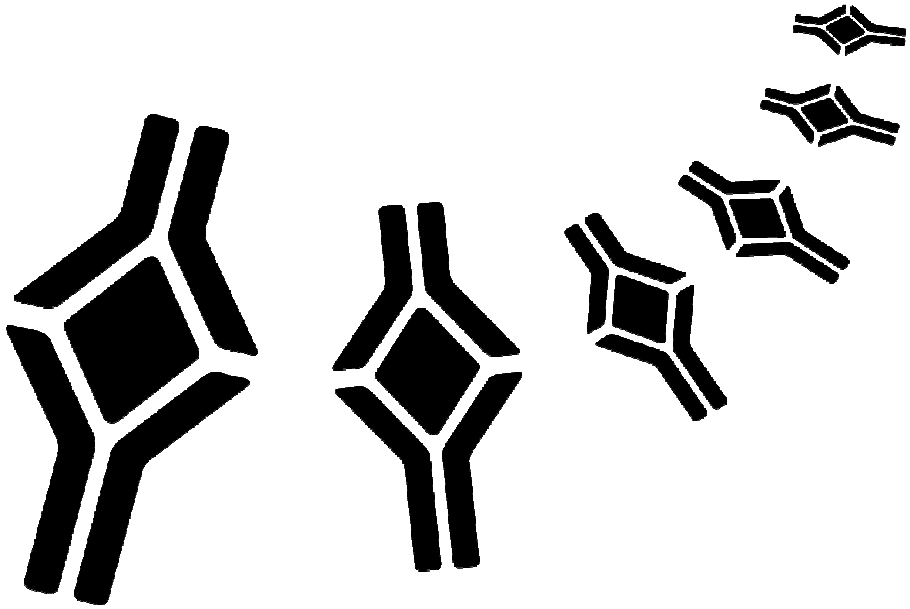
Research
and Diagnostic Products



HUMAN ADIPONECTIN ELISA, HIGH SENSITIVITY

Product Data Sheet

Cat. No.: RD191023100

European Union: IVD CE

Rest of the world:
For research use only!

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD191023100 Human Adiponectin ELISA, High Sensitivity is a sandwich enzyme immunoassay for the quantitative measurement of human adiponectin.

Features

- **European Union: for in vitro diagnostic use**
  Rest of the world: for research use only!
- The total assay time is less than 3 hours
- The kit measures total adiponectin in serum, plasma (EDTA, citrate, heparin), urine, cerebrospinal fluid (CSF)
- Assay format is 96 wells
- Quality Controls are human serum based. No animal sera are used
- 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. INTRODUCTION

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 tissue-expressed 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.

<u>Clinical application and areas of investigation:</u>
Energy metabolism and body weight regulation
Metabolic syndrome
Type 2 diabetes
Coronary artery disease
Atherosclerosis

4. TEST PRINCIPLE

In the BioVendor Human Adiponectin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human adiponectin antibody. After 60 minutes incubation and washing, polyclonal anti-human adiponectin antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured adiponectin. Following another washing step, the remaining HRP conjugate 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 proportional to the concentration of adiponectin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. 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
- 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

6. 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

7. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 ml
Set of Standards	ready to use	8 x 1 ml
Quality Control HIGH	concentrated	0.1 ml
Quality Control LOW	concentrated	0.1 ml
Dilution Buffer Conc. (10x)	concentrated	20 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. 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 5-1000 µl with disposable tips
- Multichannel pipette to deliver 100 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)

9. 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:**

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

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

Human Adiponectin Standards
The Standards are ready to use.
Stability and storage:
Opened Standards are stable 3 months when stored at 2-8°C.

- **Assay reagents supplied concentrated:**

Dilution Buffer Conc. (10x)
Dilute only required amount of Dilution Buffer Concentrate. Otherwise dilute all 20 ml of Dilution Buffer Concentrate (10x) with 180 ml of distilled water to prepare 200 ml of Dilution Buffer (1x) for use of all-wells.
Stability and storage:
The diluted Dilution Buffer is stable 1 week when stored at 2-8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2-8°C.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current Quality Control concentration!!!
Dilute Quality Control (HIGH and LOW) 10x with the Dilution Buffer just prior to the assay, e.g. 30 µl of QC + 270 µl of Dilution Buffer for duplicates. (Quality Controls are supplied diluted 30x). **It means the final dilution is 300x and the concentration of Quality Control calculated from the standard curve must be multiplied by a dilution factor of 300**.
Mix well (not to foam). Vortex is recommended. Beware of imprecision in pipetting.
Stability and storage:
Opened Quality Controls are stable 3 months when stored at 2-8°C.
Do not store the diluted Quality Controls.
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 PDS and CoA and that ELISA test was carried out properly.

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.

10. PREPARATION OF SAMPLES

The kit measures adiponectin in serum, plasma (EDTA, citrate, heparin, urine, cerebrospinal fluid (CSF), but also in breast milk.

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.

Serum and plasma samples:
Dilute serum and plasma 300x with the Dilution Buffer prior to the assay in two steps:
Dilution A (10x):
Add 10 µl of samples to 90 µl of Dilution Buffer. **Mix well** (not to foam).
Dilution B (30x):
Add 10 µl of Dilution A into 290 µl of Dilution Buffer to prepare final dilution (300x). **Mix well** (not foam).
One step-dilution can be performed (add 5 µl of samples to 1495 µl of Dilution Buffer). Beware of imprecision in pipetting and mix the samples very thoroughly!

Breast milk, urine and cerebrospinal fluid (CSF) samples:
Dilute samples 3x with Dilution Buffer just prior to the assay, e.g. add 100 µl of sample to 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam).

Stability and storage:
Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Stability of milk, urine and CSF samples have not been tested.
Do not store the diluted samples.

See Chapter 13 for 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.

11. ASSAY PROCEDURE

Adiponectin levels are significantly lower (2-3 orders of magnitude) in breast milk, urine and CSF than in serum and plasma. Therefore, different protocols have to be used.

Protocol (a) for serum and plasma samples:
Sample dilution is 300x
Standard range is 5-100 ng/ml (the Standards of 150 ng/ml and/or 2 ng/ml can be added optionally)
Incubation with Substrate Solution is 10 minutes

Protocol (b) for breast milk, urine and CSF:
Sample dilution 3x
Standard range 1-50 ng/ml
Incubation with Substrate Solution is 25-30 minutes

The other assay procedure is same for both ELISA protocols.

1. Pipet **100 µl** of Standards (5-100 ng/ml for serum and plasma samples, 1-50 ng/ml for milk, urine and CSF samples), diluted Quality Controls, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. 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.
4. Add **100 µl** of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. 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.
7. Add **100 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.
8. Incubate the plate for **10 minutes** (serum and plasma samples) or **25-30 minutes** (milk, urine and CSF samples) at room temperature (20-30°C). The incubation time may be extended [up to 20 minutes for serum and plasma samples or up to 50 minutes for milk, urine and CSF samples] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. 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 9.**

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 100**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 50**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 20**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 10**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 2**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 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.

12. 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 ng/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 Quality Controls calculated from the standard curve must be multiplied by a dilution factor of 300 and the measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because Quality Controls and samples have been diluted prior to the assay, e.g. 13.5 ng/ml (from standard curve) x 300 (dilution factor for serum and plasma samples) = 4.05 µg/ml

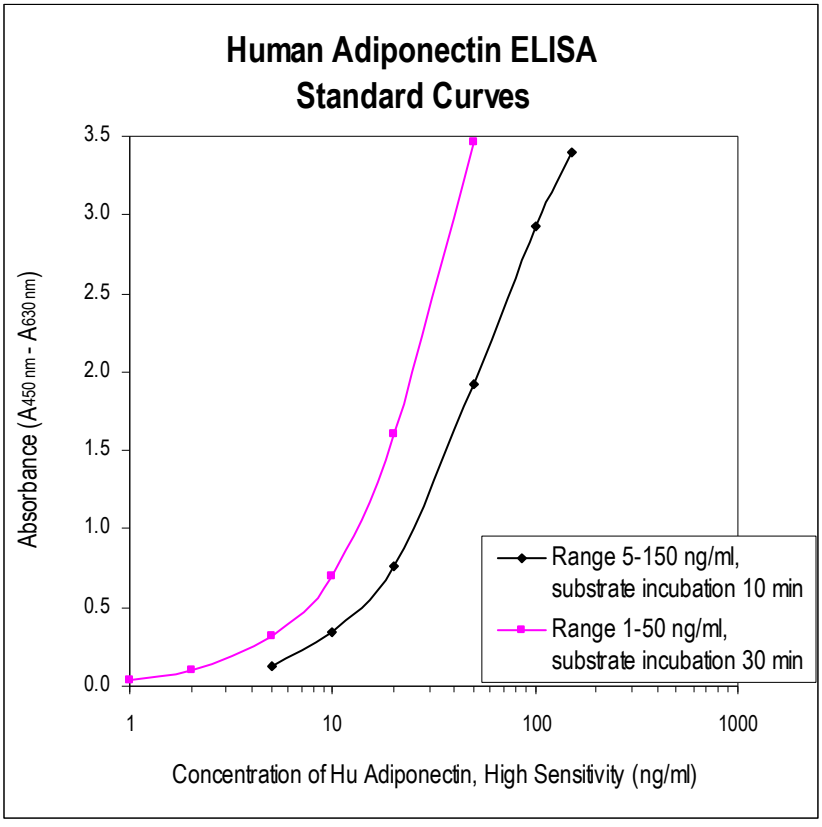


Figure 2: Typical Standard Curves for Human Adiponectin ELISA, High Sensitivity.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Adiponectin ELISA, High Sensitivity are presented in this chapter

- **Sensitivity**

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3xSD_{blank}$) is calculated from the real adiponectin values in wells and is different for two protocols described in the paragraph 11:

For **Protocol (a)** (for serum and plasma samples) recommended sample dilution 300x, calibration range 5–150 ng/ml, substrate incubation 10 min: LOD is 0.47 ng/ml.

For **Protocol (b)** (for urine and CSF samples) recommended sample dilution 3x, calibration range 1-50 ng/ml, substrate incubation 25-30 min: LOD is 0.156 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding the calibration range should be repeated with more diluted samples.
The samples with extremely high adiponectin levels can be diluted up to 2 400x. 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 (full length, mutation-modified trimer-only-forming, and globular domain) human adiponectin.

No cross-reactivity has been observed for human leptin, leptin receptor and resistin at 100 ng/ml.

Determination of adiponectin does not interfere with hemoglobin (0.25 mg/ml), bilirubin (85 umol/l) and triglycerides (2.5 mmol/l). Interference over 10% was measured at the higher concentrations.

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

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

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	6.34	0.28	4.4
2	9.41	0.31	3.3

Inter-assay (Run-to-Run) (n=9)

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	9.41	0.54	5.8
2	17.74	1.11	6.2

- **Spiking Recovery**

Samples were spiked with different amounts of human adiponectin and assayed.

For **protocol (a)** with **serum** samples:

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	4.65	-	-
	21.90	24.34	90.0
	14.40	15.57	92.5
	10.16	10.10	100.6
2	7.79	-	-
	23.87	27.48	86.9
	15.58	18.71	83.3
	11.87	13.24	89.7

For **protocol (b)** with **urine** samples:

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	15.44	-	-
	76.75	76.30	100.6
	47.53	42.07	113.0
	25.98	25.29	102.7
2	1.05	-	-
	59.91	61.91	96.8
	25.91	27.68	93.6
	12.15	10.90	111.5

For **protocol (b)** with **CSF** samples:

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	6.53	-	-
	39.46	44.03	89.6
	18.17	21.53	84.4
	13.16	14.03	93.8
2	6.67	-	-
	40.85	44.17	92.5
	19.72	21.67	91.0
	13.61	14.17	96.0

- **Linearity**

Samples were serially diluted with Dilution Buffer and assayed.

For **protocol (a)** with **serum** samples:

Sample	*Dilution*	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	-	14.21	-	-
	2x	6.51	7.11	91.6
	4x	4.05	3.55	113.9
	8x	1.73	1.78	97.3
2	-	19.98	-	-
	2x	10.51	9.99	105.2
	4x	5.40	5.00	108.1
	8x	2.35	2.50	94.1

For **protocol (b)** with **urine** samples:

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	70.56	-	-
	2x	42.42	35.28	120.2
	4x	21.00	17.64	119.0
	8x	9.18	8.82	104.1
2	-	27.08	-	-
	2x	14.83	13.54	109.5
	4x	7.35	6.77	108.6

For **protocol (b)** with **CSF** samples:

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	25.52	-	-
	2x	12.30	12.76	96.4
	4x	5.82	6.38	91.2
2	-	31.97	-	-
	2x	18.39	15.98	115.1
	4x	9.58	7.99	119.9
	8x	4.65	4.00	116.5

- **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	9.46	8.49	8.64	10.42
2	7.24	7.9	6.43	6.66
3	6.28	5.99	5.54	6.62
4	9.92	9.86	9.26	8.66
5	19.94	18.95	15.53	19.01
6	18.02	19.58	12.37	17.82
7	8.59	6.76	5.74	17.91
8	18.91	20.16	17.79	20.07
9	18.82	15.34	15.43	17.83
10	8.47	6.64	6.63	8.08
Mean (µg/ml)	**12.4**	**12.0**	**10.3**	**12.3**
Mean Plasma/Serum (%)	**-**	**96.8**	**83.6**	**99.6**
Coefficient of determination R^2	**-**	**0.96**	**0.90**	**0.98**

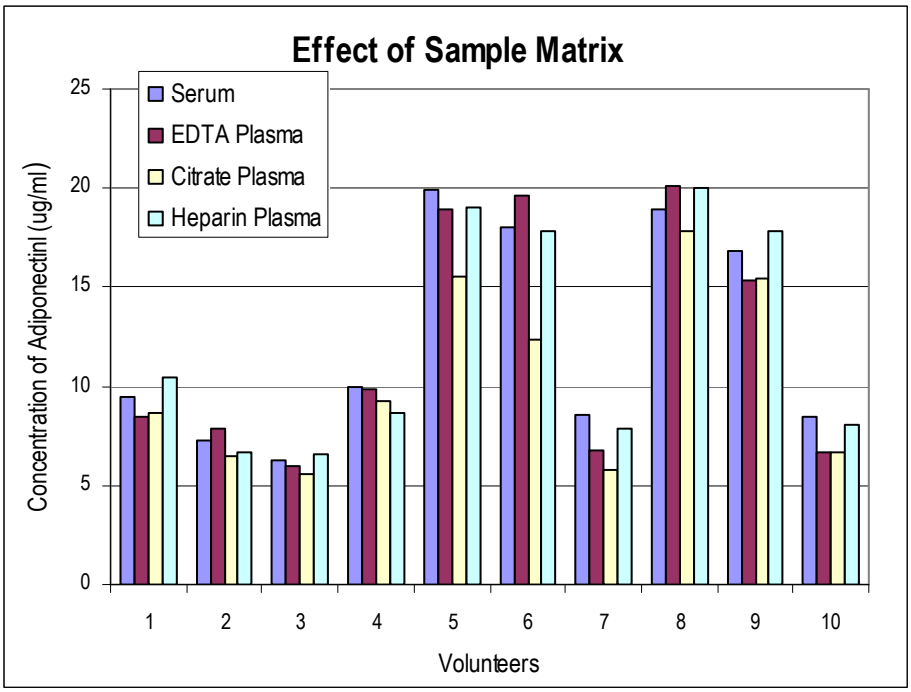


Figure 3: Adiponectin levels measured using Human Adiponectin ELISA, High Sensitivity from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **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	3.02	3.13	2.59	3.45
	2-8°C, 1 day	2.95	2.84	2.54	2.95
	2-8°C, 7 days	2.68	2.87	2.5	2.95
2	-20°C	6.77	6.82	6.22	7.06
	2-8°C, 1 day	6.77	6.60	5.83	6.69
	2-8°C, 7 days	7.11	7.06	6.06	7.04
3	-20°C	12.78	12.26	10.81	12.46
	2-8°C, 1 day	13.52	13.29	11.85	13.28
	2-8°C, 7 days	14.05	13.06	12.64	14.12

- **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	7.97	9.02	7.55	10.28
	3x	8.33	9.06	8.27	9.03
	5x	7.68	9.42	6.77	7.81
2	1x	12.92	14.60	10.87	13.31
	3x	13.34	13.32	12.61	12.78
	5x	12.38	15.11	13.31	15.78
3	1x	11.57	12.23	11.03	14.45
	3x	10.55	14.55	11.03	15.74
	5x	11.97	15.14	10.80	13.88

14. 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.

- **Reference range for serum samples**

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for adiponectin levels with the assay.

- **Tissue extract**

Adiponectin was detected in adipose tissue extracts. Concentrations of 0.2 to 2 µg/ml were found (total protein concentration 1 mg/ml).

- **Milk samples**

Adiponectin concentrations measured in breast milk samples (n = 18) were in the range of 7 – 40 ng/ml.

- **Urine samples**

Adiponectin concentrations were measured in proteinuremic urine samples (n = 10) and non-proteinuremic urine samples (n = 10). Significant differences between the two groups were observed.

Urine samples	*Sample ID*	*Adiponectin (ng/ml)*	*Mean (ng/ml)*	*SD (ng/ml)*
Proteinuremic samples	4	24.1		
	19	114.3		
	37	1.1		
	40	3.7	59.7	83.9
	41	135.1		
	50	18.5	(n=10)	(n=10)
	69	5.3		
	83	21.2		
	176	256.8		
	196	16.7		
Non-Proteinuremic samples	51	1.3		
	52	ND		
	54	19.1		
	59	ND	3.1	6.5
	73	ND		
	87	ND	(n=10)	(n=10)
	103	ND		
	128	ND		
	146	ND		
	163	10.5		

ND - Adiponectin concentrations was bellow 0.5 ng/ml (not detectable).

- **Cerebrospinal fluid samples**

Adiponectin concentrations were measured in serum and CSF samples obtained from the same persons (n = 36).

Sample ID	*Adiponectin in CSF (ng/ml)*	*Adiponectin in serum (ng/ml)*
1	17.9	13 810
3	19.8	1 727
4	84.0	9 140
5	17.7	20 080
6	8.1	11 250
11	11.4	6 300
12	200.0	11 700
13	54.0	8 180
14	70.9	15 780
15	4.8	14 870
16	5.3	13 820
23	16.4	10 070
24	3.5	9 180
25	10.8	15 480
27	26.7	20 960
29	29.0	22 900
30	4.4	11 220
31	179.9	32 540
32	13.0	7 580
33	23.6	16 320
34	172.7	8 170
35	14.1	6 940
36	27.3	10 520
41	14.9	18 930
42	5.2	7 660
43	8.1	7 460
46	4.6	12 040
48	32.6	19 620
51	17.8	11 950
52	0.0	7 370
53	30.9	33 200
54	31.2	25 650
55	7.6	13 900
56	10.4	7 530
57	12.2	22 890
58	8.9	13 920
Mean	33.3	14 340
SD	48.3	6 782
n	n = 36	n = 36

16. METHOD COMPARISON

The BioVendor's Human Adiponectin ELISA, High Sensitivity (a sandwich ELISA, RD191023100R) was compared with the BioVendor's Human Adiponectin ELISA (a competitive ELISA, RD195023100R), by measuring of 33 serum samples. The following correlation graph was obtained:

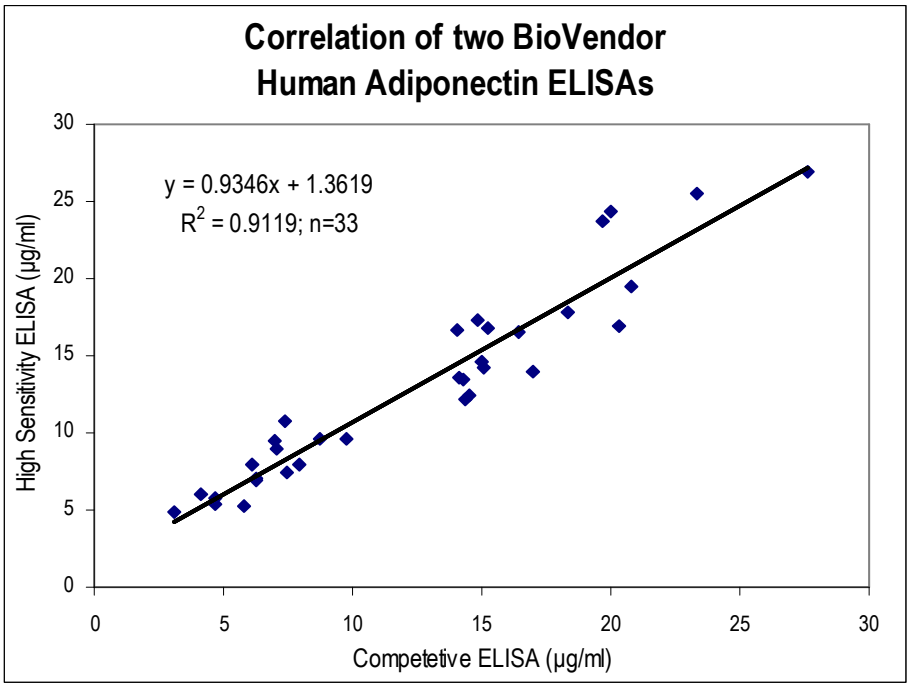


Figure 4: Method comparison.

17. 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

›› High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

18. REFERENCES

References to adiponectin:

- 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

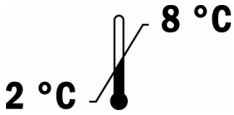
- Das K, Lin Y, Widen E, Zhang Y, Scherer PE. Chromosomal localization, expression pattern, and promoter analysis of the mouse gene encoding adipocyte-specific secretory protein Acrp30. Biochem Biophys Res Commun. 2001; 280: 1120-1129
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta 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

›› References to this product:

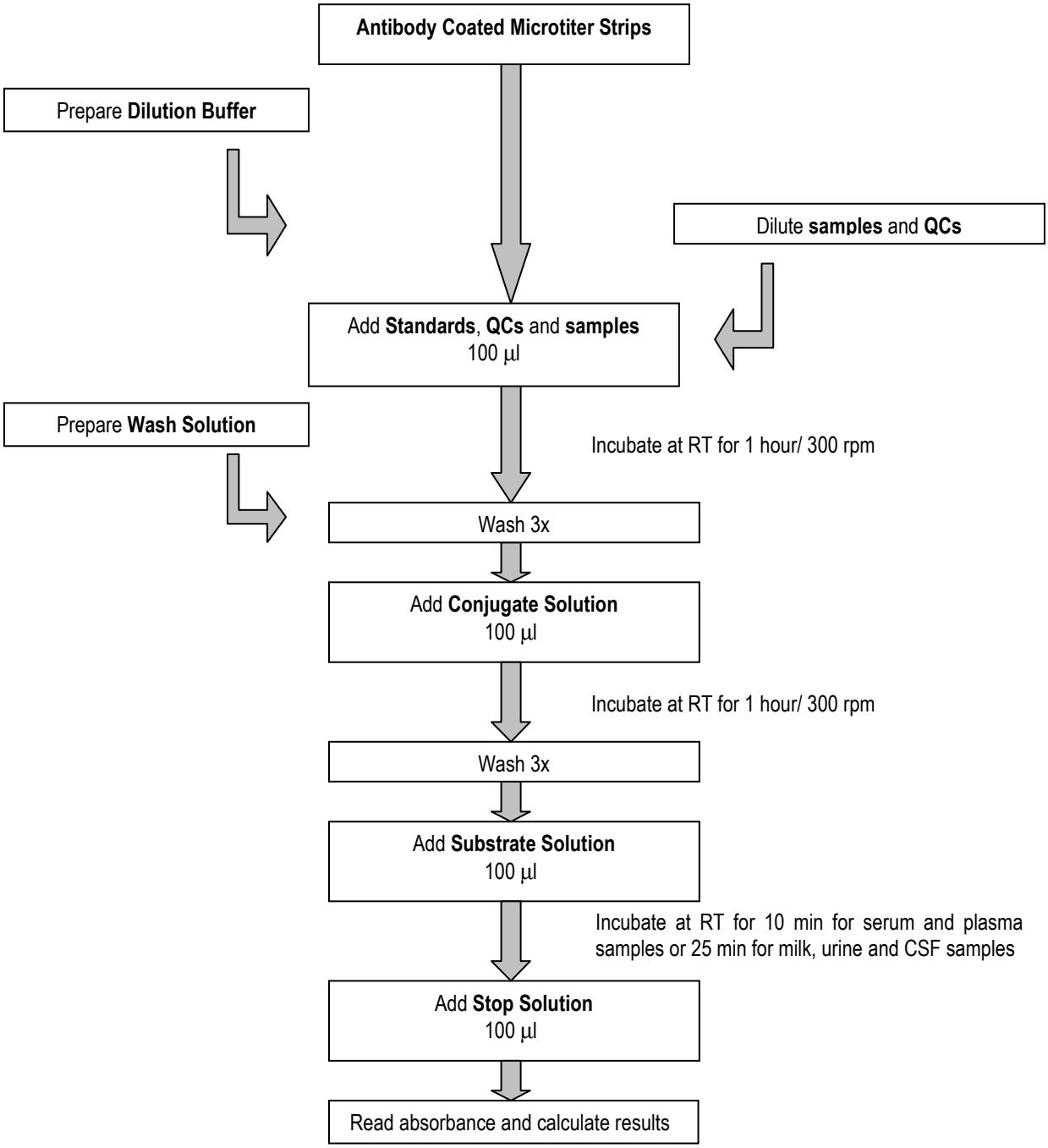
- Risch L, Saely C, Hoefle G, Rein P, Langer P, Gouya G, Marte T, Aczel S, Drexel, H: Relationship between glomerular filtration rate and the adipokines adiponectin, resistin and leptin in coronary patients with predominantly normal or mildly impaired renal function. Clin Chim Acta 376 (1-2):108-13 (2007). Epub Jul 29 (2006)
- Bronsky J, Karpisek M, Bronska E, Pechova M, Jancikova B, Kotolova H, Stejskal D, Prusa R, Nevoral J: Adiponectin, adipocyte fatty acid binding protein, and epidermal fatty acid binding protein: proteins newly identified in human breast milk. Clin Chem 52 (9):1763-70 (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. 2005, 96(7), 946-951.
- Karaduman M, Sengul A, Oktenli C, Pekel A, Yesilova Z, Musabak U, Sanisoglu SY, Gunay C, Baysan O, Kocar IH, Tatar 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). 2006, 64(2), 196-202.
- 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) (PubMed)
- Efstathiou SP, Tsioulos DI, Tsiakou AG, Gratsias YE, Pefanis AV, Mountokalakis TD: Plasma adiponectin levels and five-year survival after first-ever ischemic stroke. Stroke Sep;36(9):1915-9 (2005) (PubMed)

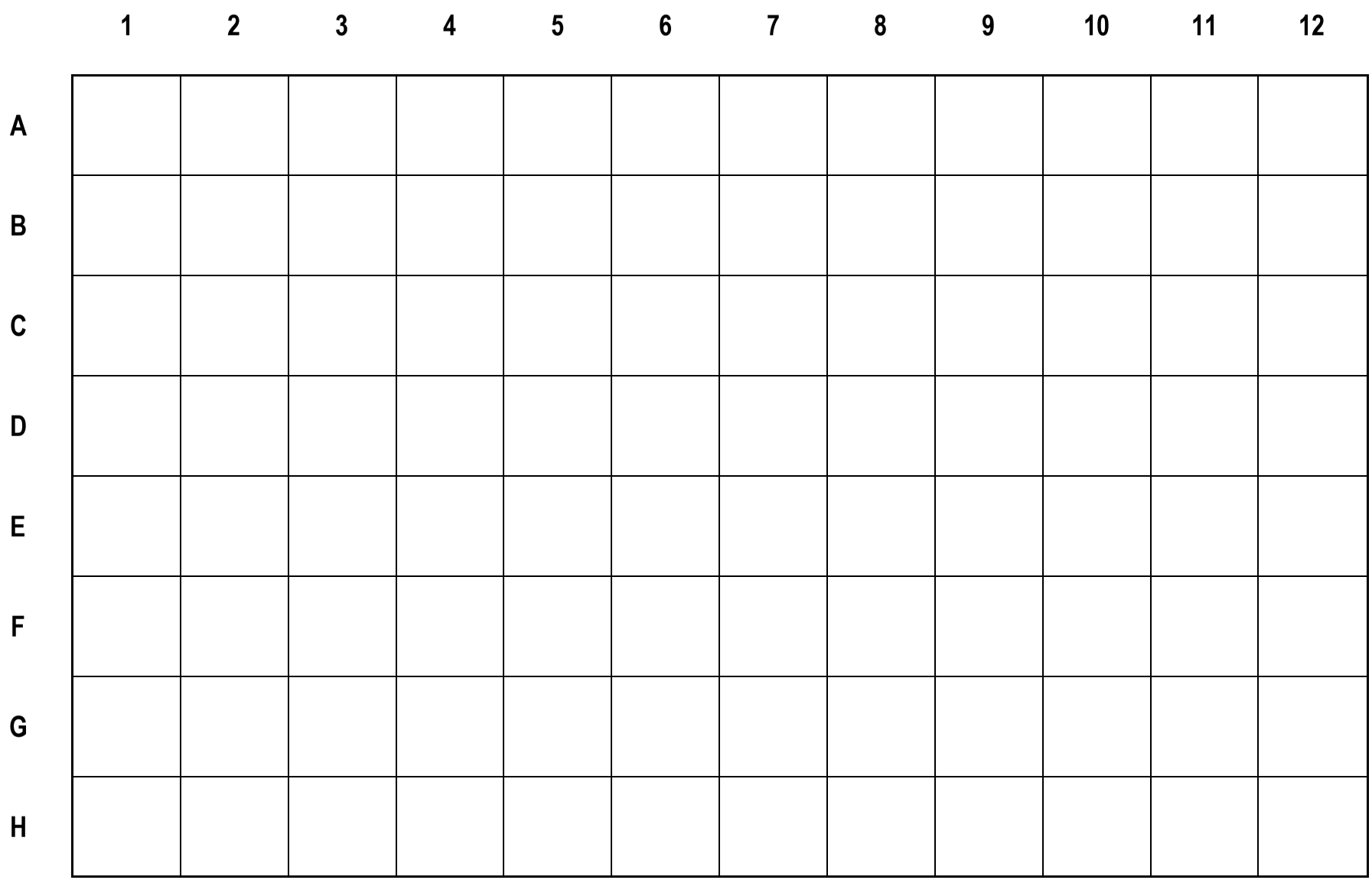
›› For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials
IVD CE	In vitro diagnostic medical device

Assay Procedure Summary

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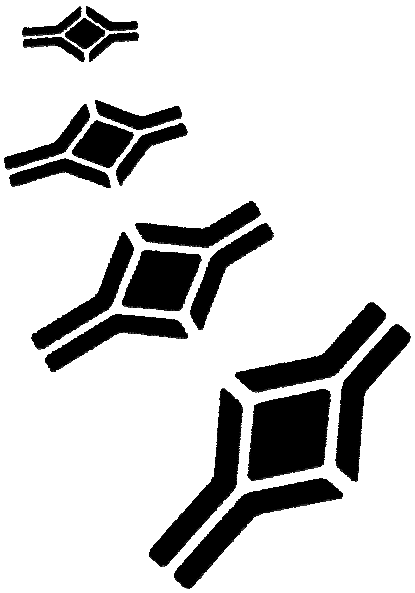

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