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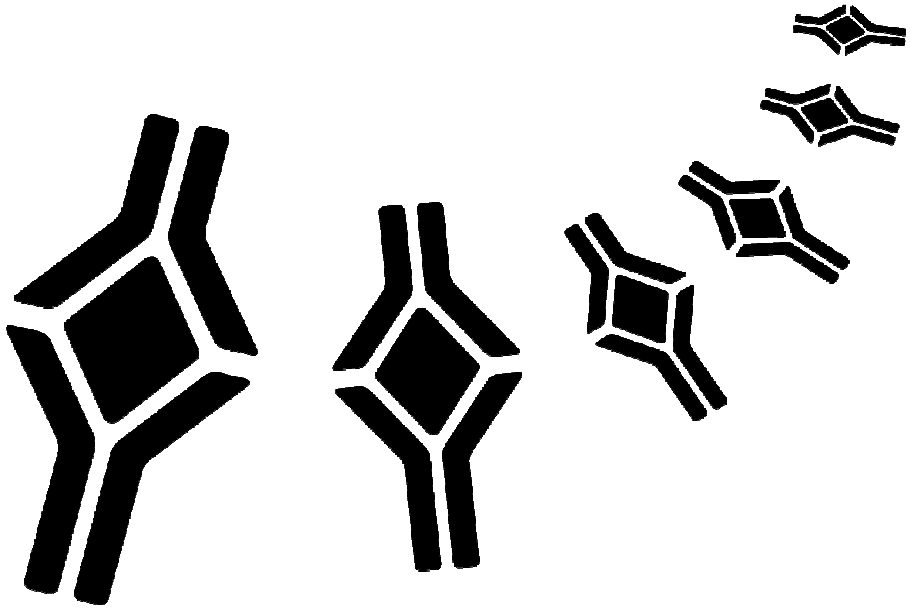


HUMAN ADIPONECTIN ELISA

Product Data Sheet

Cat. No.: RD195023100

European Union: IVD CE

Rest of the world:
For research use only!

VERSION 98 140411 15

CONTENTS

1.	INTENDED USE	3
2.	STORAGE, EXPIRATION	3
3.	INTRODUCTION	4
4.	TEST PRINCIPLE	5
5.	PRECAUTIONS	5
6.	TECHNICAL HINTS	6
7.	REAGENT SUPPLIED	6
8.	MATERIAL REQUIRED BUT NOT SUPPLIED	7
9.	PREPARATION OF REAGENTS	8
10.	PREPARATION OF SAMPLES	9
11.	ASSAY PROCEDURE	10
12.	CALCULATIONS	12
13.	PERFORMANCE CHARACTERISTICS	13
14.	DEFINITION OF THE STANDARD	17
15.	PRELIMINARY POPULATION AND CLINICAL DATA	17
16.	METHOD COMPARISON	18
17.	TROUBLESHOOTING AND FAQS	20
18.	REFERENCES	21
19.	EXPLANATION OF SYMBOLS	26

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 RD195023100 Human Adiponectin ELISA is a competitive 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 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. 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. Of particular interest 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, adiponectin has the potential to become a clinically relevant parameter to be measured routinely in subjects at risk for type 2 diabetes, atherosclerosis and the metabolic syndrome.

<u>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 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.

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
- 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

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*
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

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 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)

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:**

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.

10. 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°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
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

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: 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.

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 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.

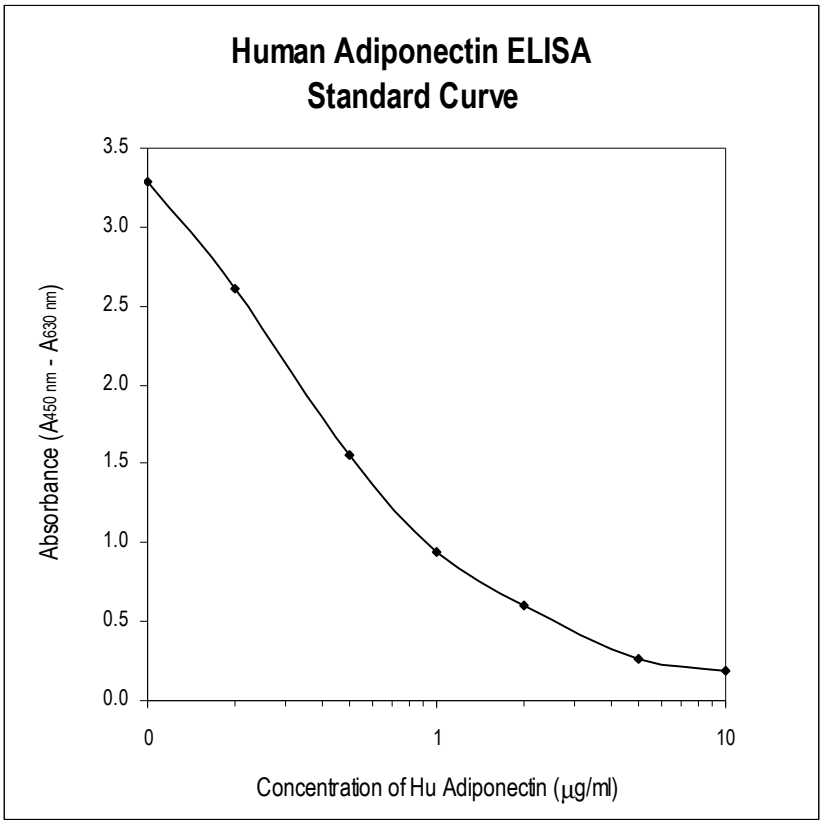


Figure 2: Typical Standard Curve for Human Adiponectin ELISA.

13. PERFORMANCE CHARACTERISTICS

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

- **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} - $3xSD_{blank}$) is calculated from the real adiponectin values in wells and is 26 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding adiponectin level of 100 µg/ml should be repeated with more diluted samples (e.g. 60x). 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, mutation-modified trimer-only-forming protein, and globular domain).
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. No interfererence has been observed for hemoglobin (5 mg/ml), bilirubin-mixed isomers (0.4 mg/ml) and triglycerides (0.25 mg/ml).

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	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
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	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

- **Spiking Recovery**

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

Sample	***O**bserved (µg/ml)*	***E**xpected (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

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (µg/ml)*	***E**xpected (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

- **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**

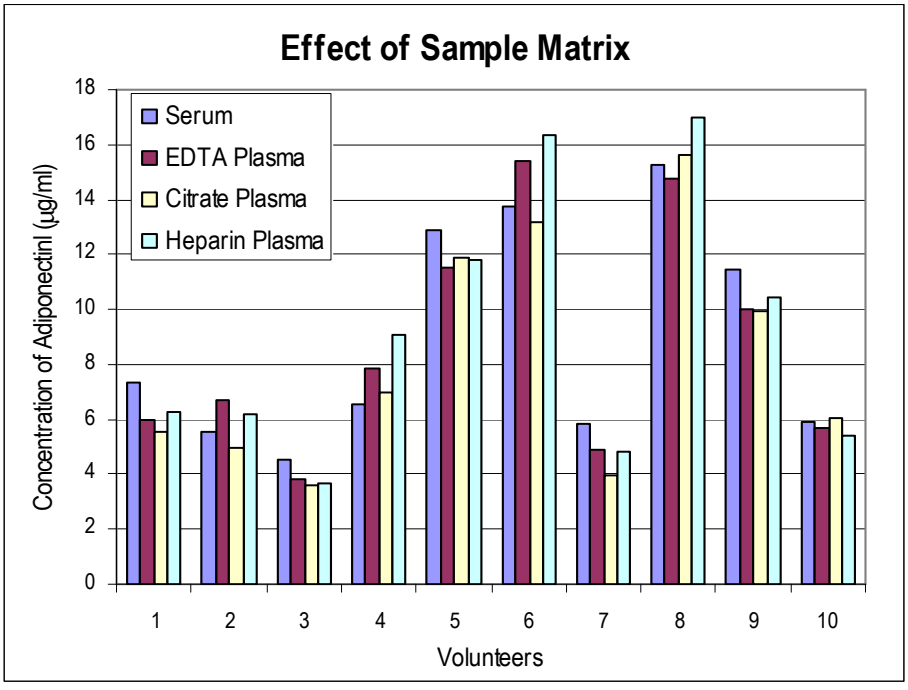


Figure 3: Adiponectin levels measured using Human Adiponectin ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **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 (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

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.

15. PRELIMINARY POPULATION AND CLINICAL DATA

- **Normal Values**

The following results were obtained when serum samples from 335 healthy donors were assayed with BioVendor's Human Adiponectin ELISA.

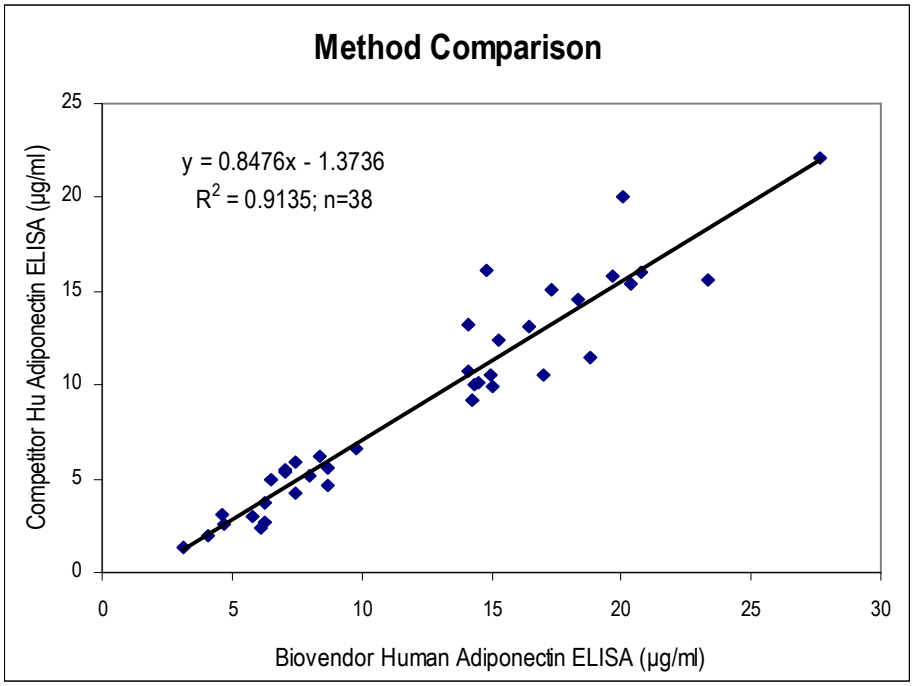
Gender	*BMI (kg/m²)*	*n*	*Mean (µg/ml)*	*SD (µg/ml)*
Men	< 25	41	10.9	4.0
	25-30	52	8.8	4.0
	> 30	23	8.3	2.8
	total	115	9.5	3.9
Women	< 25	92	13.6	5.4
	25-30	56	13.9	8.6
	> 30	57	11.4	3.8
	total	220	13.2	6.1

- **Reference range**

The data quoted in these instructions should be used for guidance only. 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.

16. METHOD COMPARISON

The BioVendor Human Adiponectin ELISA was compared to the other commercial immunoassays, by measuring of 38 serum samples, in two different ELISA. The following correlation graphs were obtained:



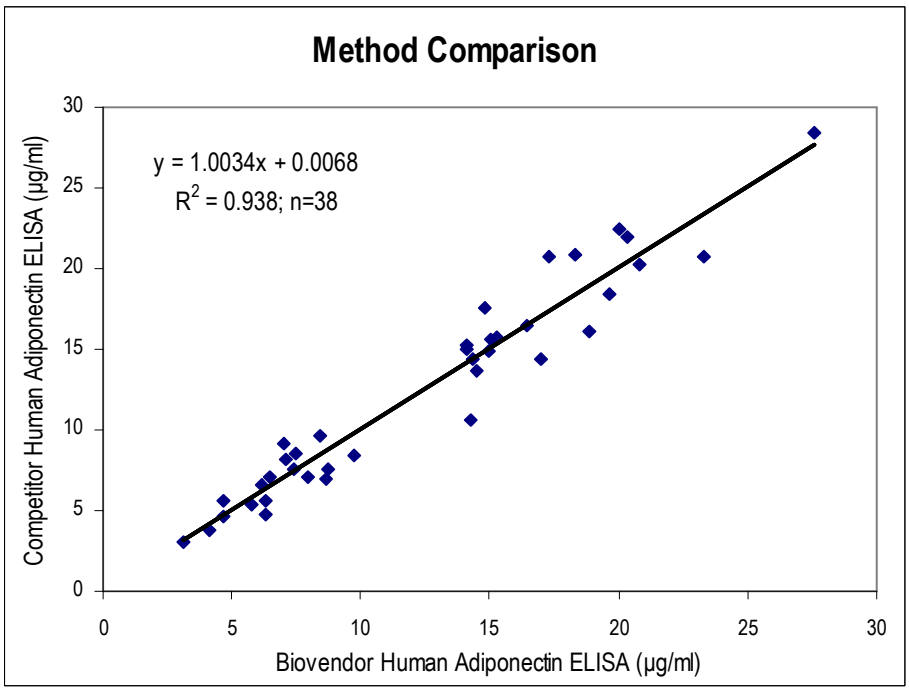


Figure 4: Method comparison.

The BioVendor's Human Adiponectin ELISA, High Sensitivity (a sandwich ELISA, RD191023100) was compared with the BioVendor's Human Adiponectin ELISA (a competitive ELISA, RD195023100), by measuring of 33 serum samples. The following correlation graph was obtained:

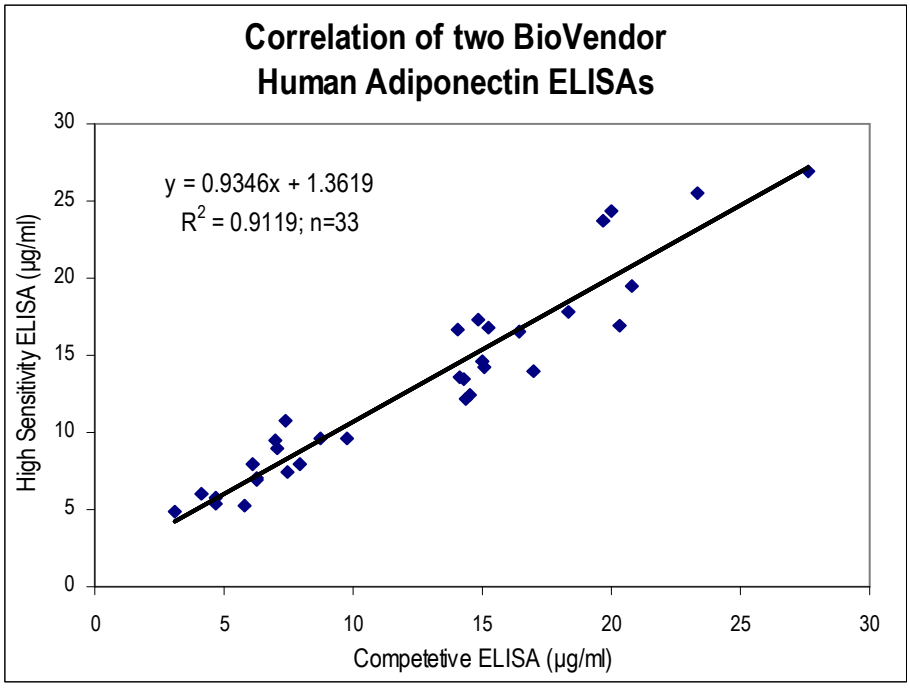


Figure 5: Method comparison with the other ELISA of the same company.

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

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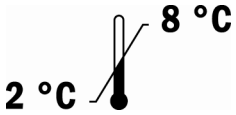
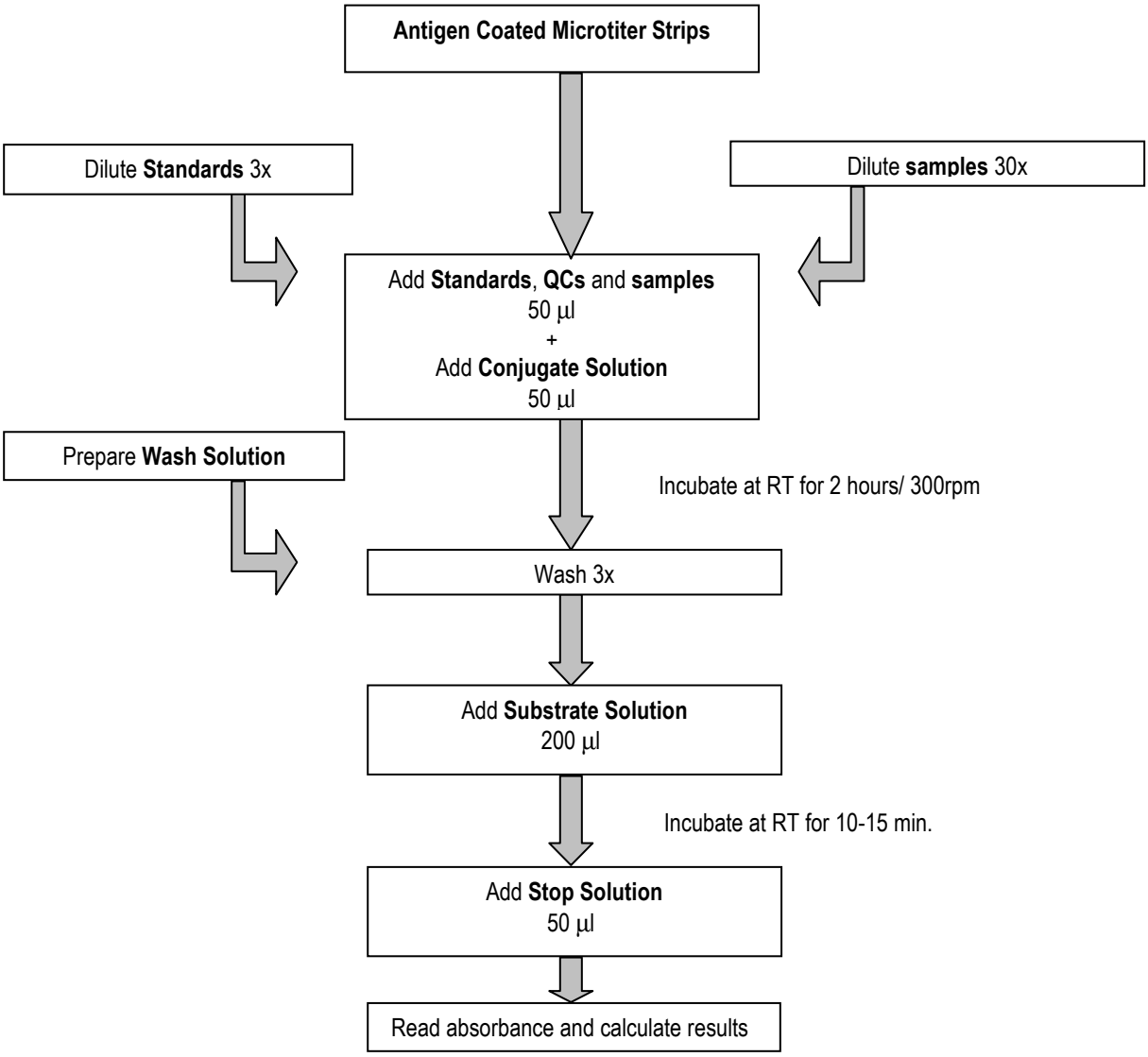
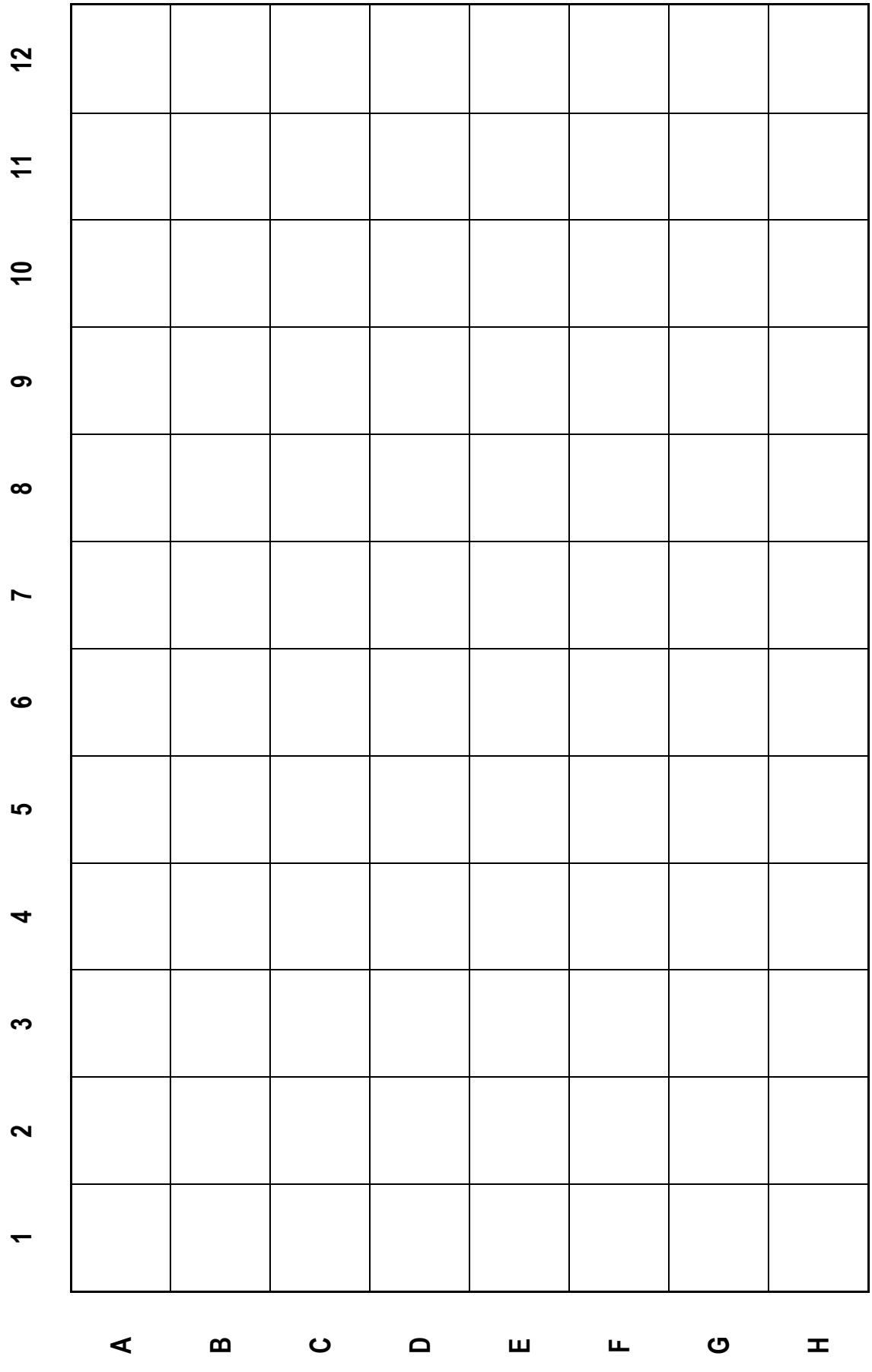
19. EXPLANATION OF SYMBOLS

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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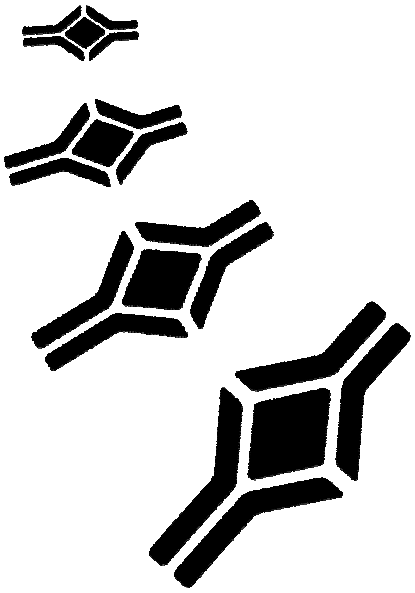

HEADQUARTERS: **BioVendor - Laboratorní medicína a.s.**	**Karasek 1767/1**	**621 00 Brno** **CZECH REPUBLIC**	**Phone: +420-549-124-185** **Fax: +420-549-211-460**	**E-mail: info@biovendor.com** **Web: www.biovendor.com**
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	1463 Sand Hill Road Suite 227	Candler, NC 28715 USA	Phone: +1-828-670-7807 +1-800-404-7807 Fax: +1-828-670-7809	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2917, 29/F R & F Ying Feng Plaza, No.2 Huaqiang road	Pearl River New Town Guang Zhou, CHINA	Phone: +86-20-38065519 Fax: +86-20-38065529	E-mail: infoCN@biovendor.com