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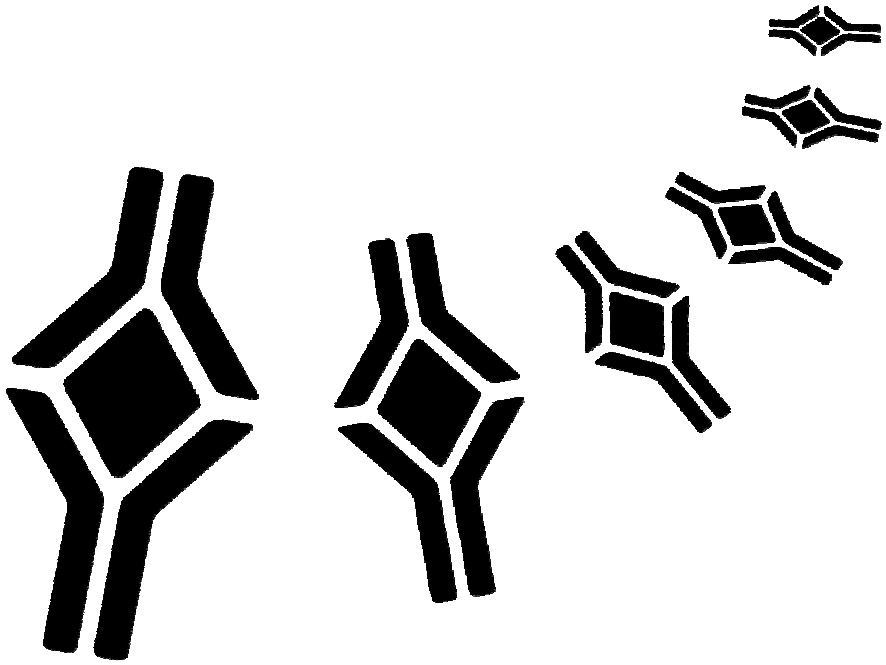
Research
and Diagnostic Products



MOUSE ADIPONECTIN ELISA

Product Data Sheet

Cat. No.: RD293023100R

For Research Use Only

VERSION 98 290311 15

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 RD293023100R Mouse Adiponectin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of mouse adiponectin.

Features

- **It is intended for research use only**
- The total assay time is less than 2.5 hours
- The kit measures total mouse adiponectin in serum
- Assay format is 96 wells
- Quality Controls are mouse serum based
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

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 (adipokine). 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. 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. A reduction in adiponectin serum levels is accompanied by insulin resistance states, such as obesity and type 2 diabetes mellitus. It is also reported in patients with coronary artery disease. Increased adiponectin levels are associated with type 1 diabetes mellitus, anorexia nervosa and chronic renal failure. Adiponectin concentrations correlate negatively with glucose, insulin, triglyceride concentrations and body mass index and positively with high-density lipoprotein-cholesterol levels and insulin-stimulated glucose disposal.

Adiponectin has been shown to increase insulin sensitivity and decrease plasma glucose by increasing tissue fat oxidation. It inhibits the inflammatory processes of atherosclerosis suppressing the expression of adhesion and cytokine molecules in vascular endothelial cells and macrophages, respectively. This 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.

<u>Areas of investigation:</u>
Energy metabolism and weight regulation
Coronary artery disease
Chronic renal failure

4. TEST PRINCIPLE

In the BioVendor Mouse Adiponectin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-mouse adiponectin antibody. After 60 minutes incubation and washing, polyclonal anti-mouse adiponectin antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured mouse 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 mouse 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 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*
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer Conc. (10x)	concentrated	22 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 10-1000 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 desicant 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.

- **Assay reagents supplied concentrated or lyophilized:**

Dilution Buffer Conc. (10x)
Dilute Dilution Buffer Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Prepare only required amount of Dilution Buffer. Example: 22 ml of Dilution Buffer Concentrate (10x) + 198 ml of distilled water for use of all 96-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.

Mouse Adiponectin Master Standard
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the mouse adiponectin in the stock solution is **8 ng/ml**.
Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	8 ng/ml
0.5 ml of stock	0.5 ml	4 ng/ml
0.5 ml of 4 ng/ml	0.5 ml	2 ng/ml
0.5 ml of 2 ng/ml	0.5 ml	1ng/ml
0.5 ml of 1 ng/ml	0.5 ml	0.5 ng/ml
0.5 ml of 0.5 ng/ml	0.5 ml	0.25 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Do not store the Standard stock solution and set of standards.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with 1 ml of Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Reconstituted Quality Controls are ready to use, do not dilute them.
Stability and storage:
Do not store the reconstituted Quality Controls.

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 mouse adiponectin in serum.

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 10 000x with Dilution Buffer just prior to the assay in two steps as follows:
Dilution A (100x):
Add 10 µl of sample into 990 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.
Dilution B (100x):
Add 10 µl of Dilution A into 990 µl of Dilution Buffer to prepare final dilution (10 000x). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum samples when stored at 2-8°C and effect of freezing/thawing on the concentration of mouse 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 **100 µl** of each individual concentration of Standards, 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** 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.
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 mouse 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 8**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 4**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 2**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 1**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.25**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC HIGH**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC LOW**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

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 mouse 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 samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 2.65 ng/ml (from standard curve) x 10 000 (dilution factor) = 26.5 μg/ml.

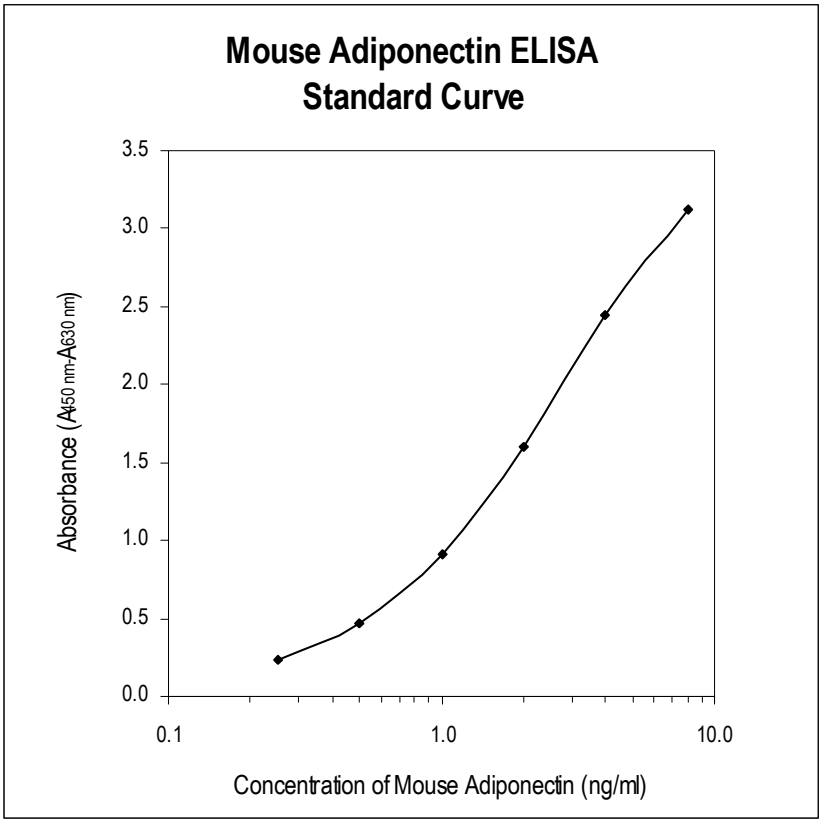


Figure 2: Typical Standard Curve for Mouse Adiponectin ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Mouse Adiponectin ELISA 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 0.1 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding adiponectin level of 8 ng/ml should be repeated with more diluted samples (e.g. 20 000x). Dilution factor needs to be taken into consideration in calculating the adiponectin concentration.

- **Specificity**

The antibodies used in this ELISA are specific for mouse adiponectin protein with no detectable crossreactivities to mouse cytokines: RELM-α, RELM-β, leptin, leptin receptor, resistin; as well as for rat leptin and human adiponectin at 100 ng/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
Human	no
Monkey	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	27.1	0.6	2.3
2	14.9	0.4	2.7

Inter-assay (Run-to-Run) (n=5)

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	25.7	1.2	4.7
2	14.6	0.6	3.8

- **Spiking Recovery**

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

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	9.0	-	-
	13.4	14.9	95.7
	18.2	19.0	95.8
	26.5	29.0	91.4
2	20.0	-	-
	25.2	25.0	100.8
	30.9	30.0	103.0
	39.4	40.0	98.5

- **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	-	24.8	-	-
	2x	11.5	12.4	92.7
	4x	5.1	6.2	82.3
	8x	2.6	3.1	83.9
2	-	52.6	-	-
	2x	25.5	26.3	97.0
	4x	12.2	13.15	92.8
	8x	5.3	6.58	80.7

- **Stability of samples stored at 2-8°C**

Samples should be stored at –20°C. However, no decline in concentration of mouse adiponectin was observed in serum samples after 14 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)	*(%)*
1	-20°C	22.1	100.0
	2-8°C, 1 day	21.1	95.2
	2-8°C, 7 days	21.0	94.8
	2-8°C, 14 days	21.4	96.7
2	-20°C	15.2	100.0
	2-8°C, 1 day	14.2	93.0
	2-8°C, 7 days	15.7	103.3
	2-8°C, 14 days	14.9	97.4
3	-20°C	16.3	100.0
	2-8°C, 1 day	15.1	92.5
	2-8°C, 7 days	16.3	99.7
	2-8°C, 14 days	17.2	105.6

- **Effect of Freezing/Thawing**

No decline was observed in concentration of mouse adiponectin in serum 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)	*(%)*
1	0	12.5	100.0
	1x	12.4	99.4
	3x	12.2	97.8
	5x	12.4	99.5
2	0	59.8	100.0
	1x	62.1	103.9
	3x	55.9	93.5
	5x	56.3	94.3
3	0	48.8	100.0
	1x	54.2	111.0
	3x	52.5	107.6
	5x	49.5	101.5

14. DEFINITION OF THE STANDARD

A recombinant protein is used as the standard. The recombinant adiponectin is a mammalian HEK293 cell-expressed protein, naturally forming LMW, MMW and HMW oligomers. The oligomer distribution is similar to that observed in mouse blood.

15. PRELIMINARY POPULATION AND CLINICAL DATA

- **Normal values**

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

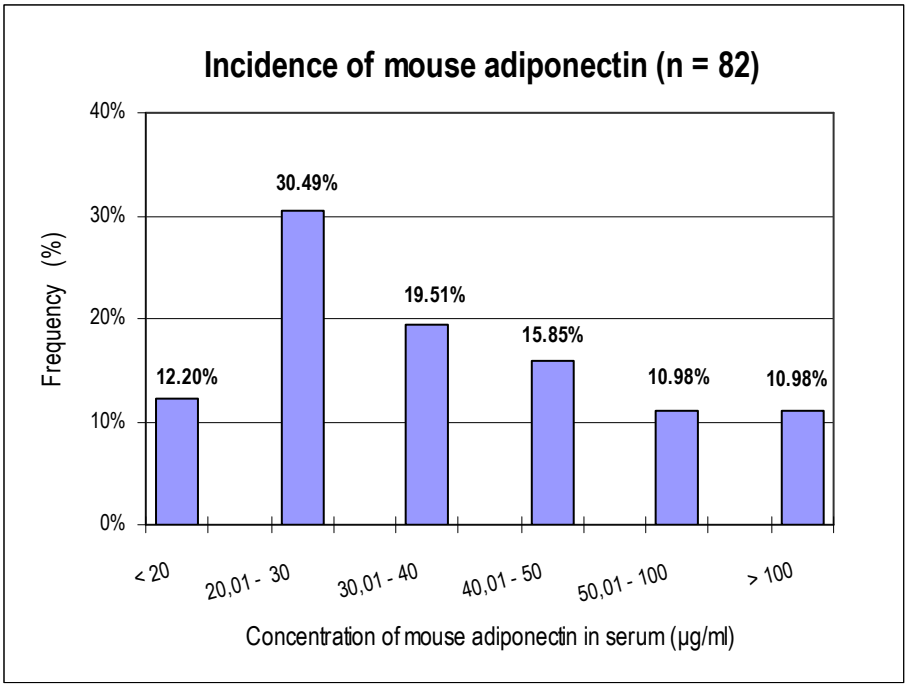


Figure 3: Normal values.

- **Reference range**

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory includes its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for mouse adiponectin levels with the assay.

16. METHOD COMPARISON

The BioVendor Mouse Adiponectin ELISA was not compared to the other commercial immunoassays.

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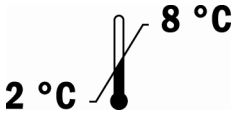
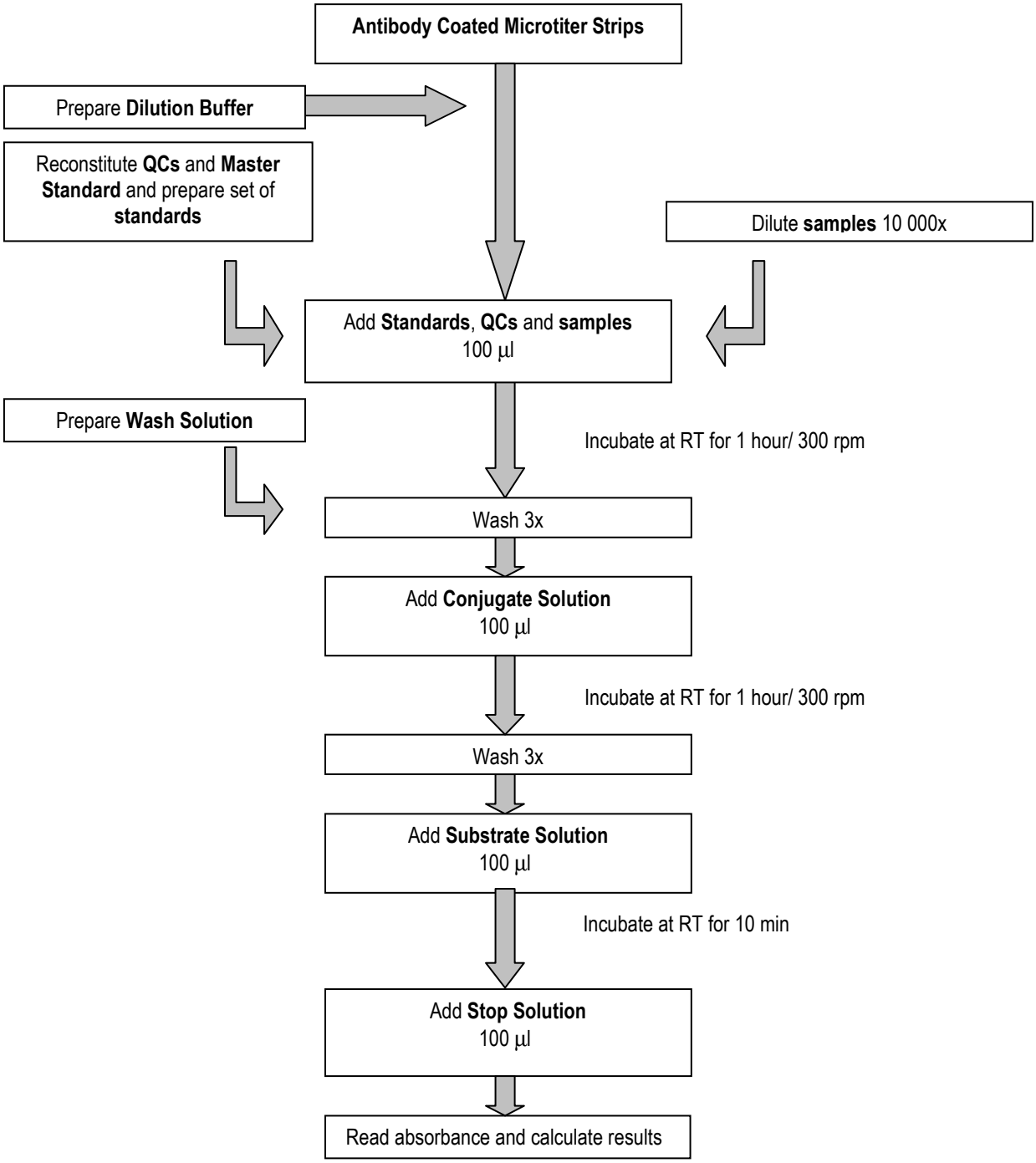
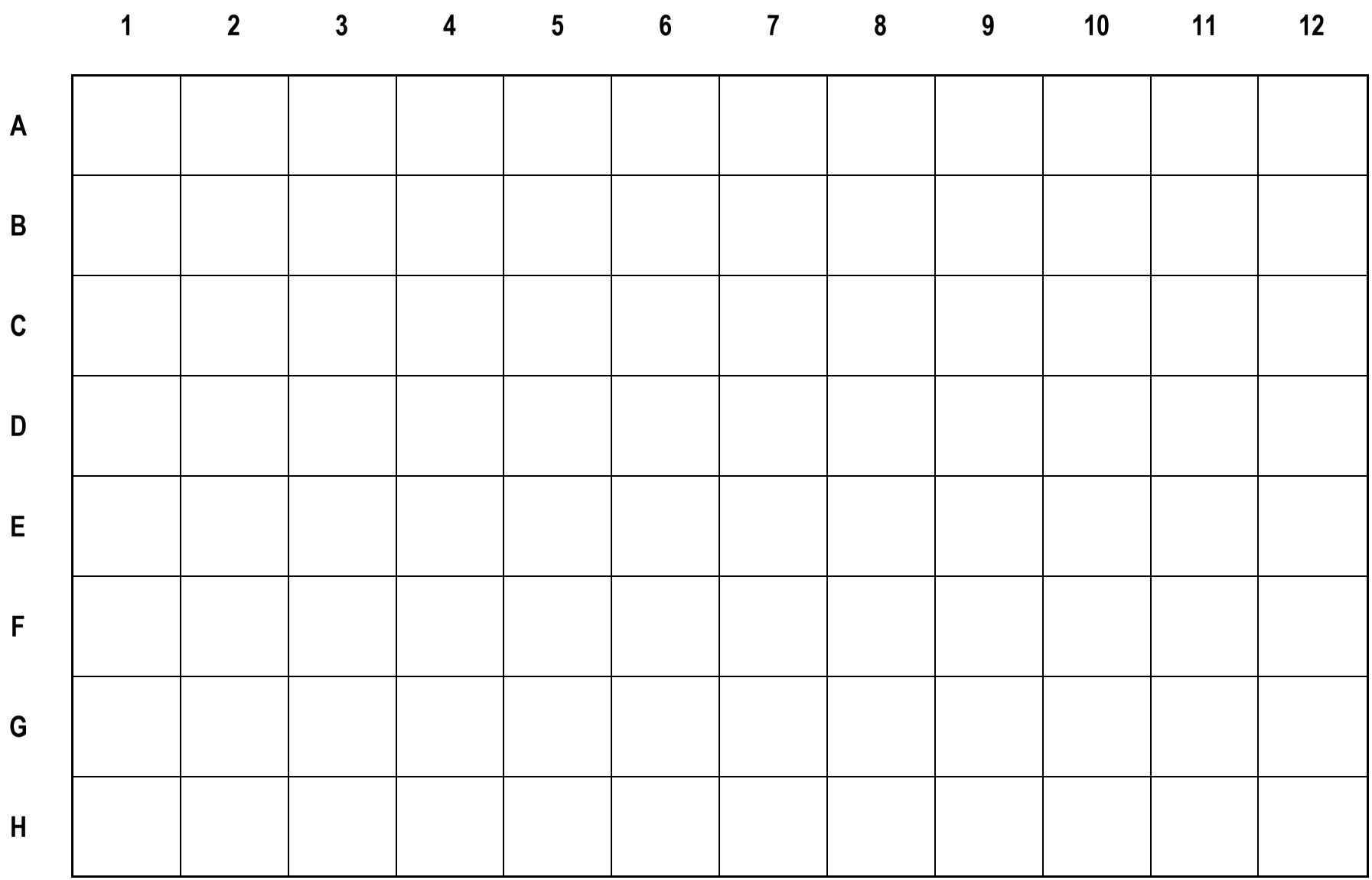
›› For more references on this product see our WebPages at www.biovendor.com

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

Assay Procedure Summary

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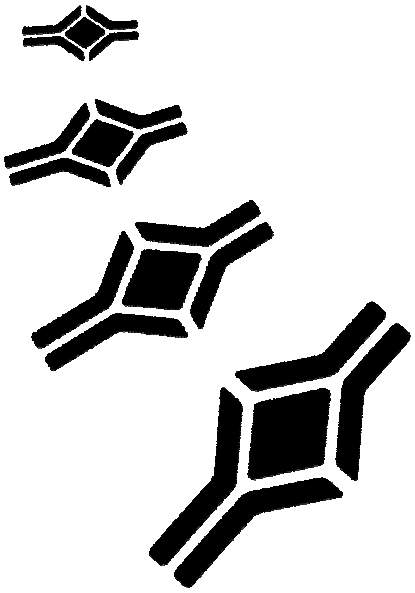

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