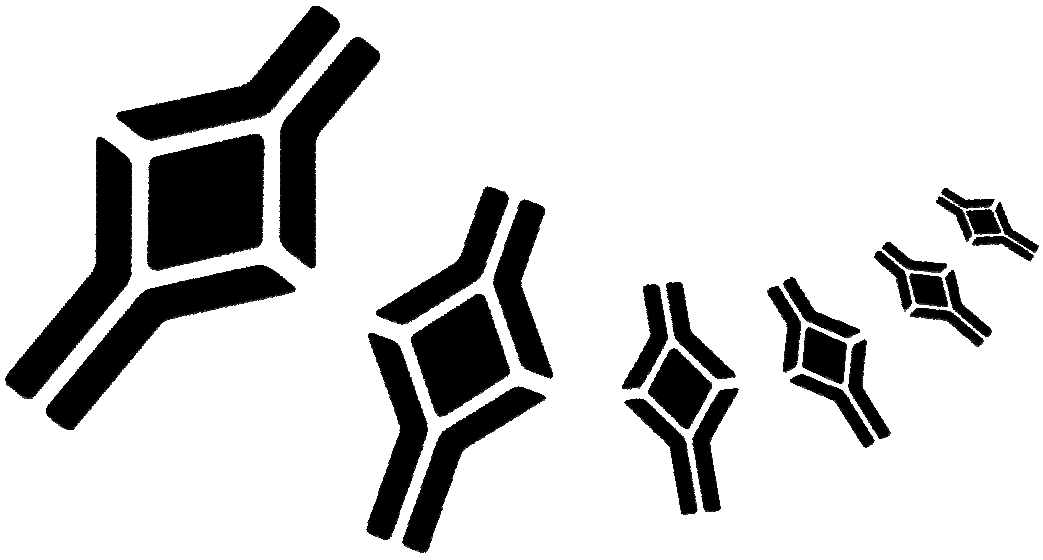
BioVendor
Research
and Diagnostic Products



MOUSE RESISTIN ELISA

Product Data Sheet

Cat. No.: RD293016100R

For Research Use Only

VERSION 03 290909 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 RD293016100R Mouse Resistin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of mouse resistin.

Features

- **It is intended for research use only.**
- The total assay time is less than 4 hours.
- The kit measures mouse resistin in serum, plasma (EDTA, citrate) and tissue culture medium.
- 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

Resistin, a product of the RSTN gene, is a peptide hormone belonging to the class of cysteine-rich secreted proteins (monomeric peptide contains 11 cysteine residues) referred to as the RELM family, and is also described as ADSF (Adipose Tissue-Specific Secretory Factor) or FIZZ3 (Found in Inflammatory Zone 3). Mouse Resistin is expressed as a 114 amino acid prepeptide; its hydrofobic N-terminal 20 amino acid signal peptide is cleaved before its secretion. Mouse Resistin circulates in blood as a homodimeric protein consisting of two 94 amino acid polypeptides, which are disulfide-linked via Cys26.
Resistin may be an important link between obesity and insulin resistance. Mouse Resistin, specifically produced and secreted by adipocyte, acts on skeletal muscle myocytes, hepatocytes and adipocytes themselves so that it reduces their sensitivity to insulin. Steppan et al. have suggested that resistin suppressed the ability of insulin to stimulate glucose uptake. They have also suggested that resistin was present at elevated levels in blood of obese mice, and was down regulated by fasting and by antidiabetic drugs. Way et al., on the other hand, have found that resistin expression is severely suppressed in obesity and is stimulated by several antidiabetic drugs.
Other studies have shown that mouse resistin increases during the differentiation of adipocytes, but it also seems to inhibit adipogenesis. In contrast, the human adipogenic differentiation is likely to be associated with a down regulation of resistin gene expression.

Areas of investigation:
Energy metabolism and body weight regulation

4. TEST PRINCIPLE

In the BioVendor Mouse Resistin ELISA, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with polyclonal anti-mouse resistin antibody. After 60 minutes incubation and washing, biotin-labelled polyclonal anti-mouse resistin antibody is added and incubated for 60 minutes with captured resistin. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining 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 resistin. 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.**
- 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
Biotin Labelled Antibody	ready to use	13 ml
Streptavidin -HRP Conjugate	ready to use	13 ml
Set of Standards	ready to use	7 x 0.05 ml
Quality Control	lyophilized	1 vial
Dilution Buffer Conc. (5x)	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 550-650nm)
- 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 stored at 2-8°C and protected from the moisture.

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

Mouse Resistin Standards
Dilute each concentration of Standard 100x with Dilution Buffer just prior to the assay, e.g.10 µl of Standard + 990 µl of Dilution Buffer. Mix well (not foam). Vortex is recommended.
Stability and storage:
Opened Standards are stable 3 months when stored at 2-8°C.
Do not store the diluted Standard solutions.

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

Dilution Buffer Concentrate (5x)
Dilute Dilution Buffer Concentrate (5x) five-fold in distilled water to prepare a 1x working solution. Example: 22 ml of Dilution Buffer Concentrate (5x) + 88 ml of distilled water for use of all 96-wells.
Stability and storage:
The diluted Dilution Buffer is stable 1 month when stored at 2-8°C. Opened Dilution Buffer Concentrate (5x) is stable 3 months when stored at 2-8°C.

Quality Control

Reconstitute Quality Control with **50 µl** of deionized (distilled) water. Let it dissolve at least 15 minutes with occasionally gently shaking (not to foam).

Dilute the reconstituted Quality Control 100x with Dilution Buffer just prior to the assay, e.g. 10 µl of Quality Control + 990 µl of Dilution Buffer. Mix well (not foam). Vortex is recommended.

Stability and storage:

The reconstituted Quality Control must be used immediately or aliquoted and frozen at -20°C for 3 month. Avoid repeated freeze/thaw cycles.

Do not store the diluted Quality Control.

Wash Solution Concentrate (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 resistin in serum, plasma or tissue culture medium.

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 100x with Dilution Buffer just prior to the assay, e.g.10 µl of sample + 990 µl of Dilution Buffer for duplicates. Mix well (not 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 effect of sample matrix (serum/plasma) on the concentration of resistin.

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 diluted Standards, Quality Controls, Dilution Buffer (=Blank) and 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 Biotin Labelled Antibody 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 Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. 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.
10. 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.
11. 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.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. 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.

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 resistin 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	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 50	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 20	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 10	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard 5	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard 2	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	Standard 1	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	Blank	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 resistin 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.

Samples, Quality control and Standards are all diluted 100x prior to the analysis, so there is no need to take this dilution factor into account.

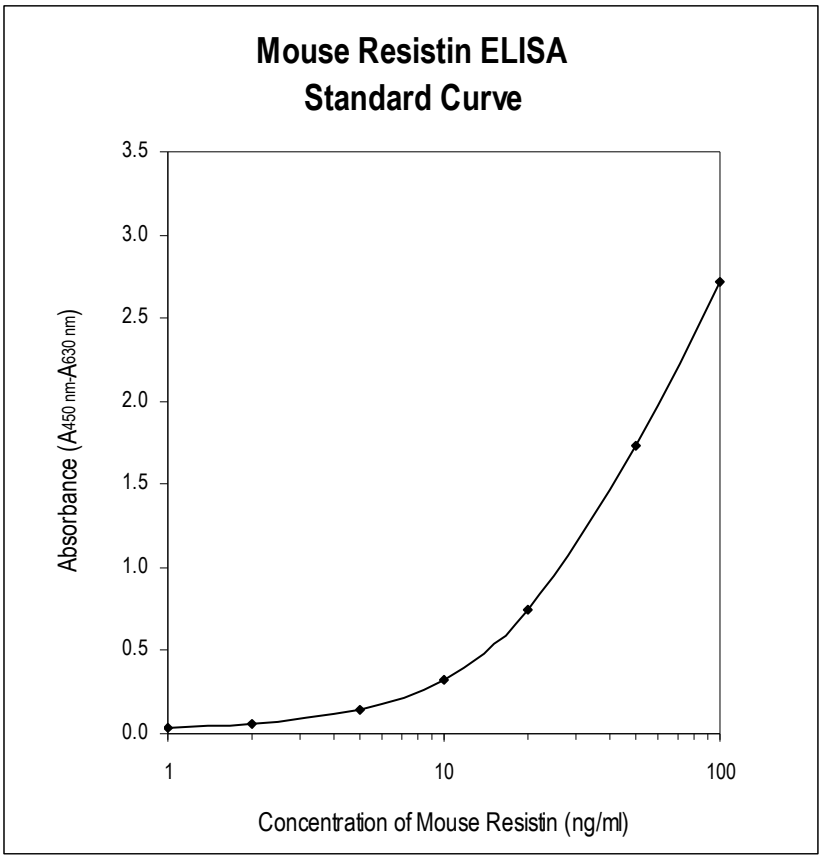


Figure 2: Typical Standard Curve for Mouse Resistin ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Mouse Resistin 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 resistin values in wells and is 5 pg/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding resistin level of 100 ng/ml should be repeated with more diluted samples (e.g. 200x). Dilution factor (e.g. 2) needs to be taken into consideration in calculating the resistin concentration.

- **Specificity**

The assay recognizes natural and recombinant mouse resistin. No *cross-reactivity* has been observed for mouse cytokines: RELM-α, RELM-β, leptin, leptin receptor and 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	yes
Horse	no
Monkey	no
Mouse	yes
Pig	no
Rabbit	no
Rat	no
Sheep	no

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	20.82	0.99	4.8
2	48.44	2.09	4.3

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	51.80	2.90	5.6
2	82.12	4.29	5.3

- **Spiking Recovery**

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

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	6.22	-	-
	10.76	11.22	96
	16.00	16.22	99
	24.06	26.22	92
2	24.90	-	-
	31.56	29.90	106
	36.82	34.90	106
	46.00	44.90	102

- **Linearity**

Serum samples (100x diluted) were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	47.03	-	-
	2x	24.00	23.52	102
	4x	11.98	11.76	102
	8x	6.44	5.88	110
2	-	78.11	-	-
	2x	39.45	39.06	101
	4x	19.96	19.53	102
	8x	9.94	9.76	102

- **Effect of sample matrix**

Citrate and EDTA plasmas were compared to respective serum samples obtained from healthy BALB/c mice. (n = 8) in the same time.

Sample (n = 8)	*Mean Mouse Resistin (ng/ml)*	*Plasma/Serum ± SD*
Serum	23.96	-
Citrate Plasma	22.10	92 ± 9 %
EDTA Plasma	20.57	86 ± 7 %

14. DEFINITION OF THE STANDARD

A recombinant protein is used as the standard. The recombinant mouse resistin is a 20.2 kDa dimeric protein consisting of two 94 amino acid polypeptide chains which are disulfide-linked.

15. PRELIMINARY POPULATION AND CLINICAL DATA

- **Normal values**

The following values were obtained when 61 sera from healthy BALB/c mice were assayed:

Mouse (BALB/c) *(n=61)*	***Mouse Resistin*** *(ng/ml)*
Mean	27.1
Median	26.7
Normal Range (Mean± 2*SD)	7.5 – 46.6

- **Reference range**

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

16. METHOD COMPARISON

BioVendor Mouse Resistin ELISA has not been compared to any other immunoassay.

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 Mouse Resistin ELISA:

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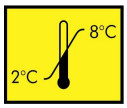
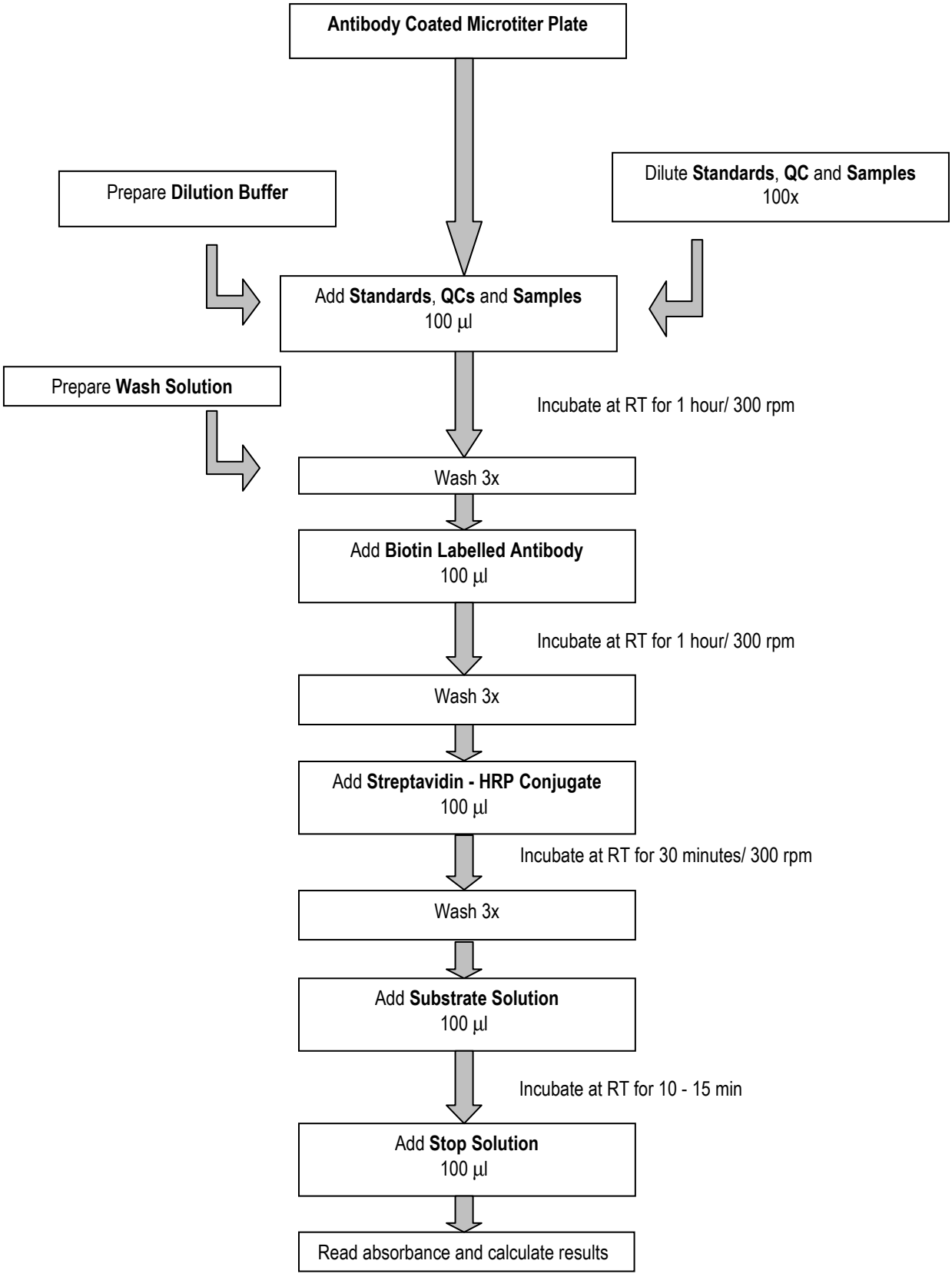
- Juan CC. at al.: Suppressed Gene Expression of Adipocyte Resistin in an Insulin-resistant Rat Model Probably by Elevated Free Fatty Acids. *Biochemical and Biophysical Research Communications*. **289**, 1328-1333, (2001)

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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NOTES

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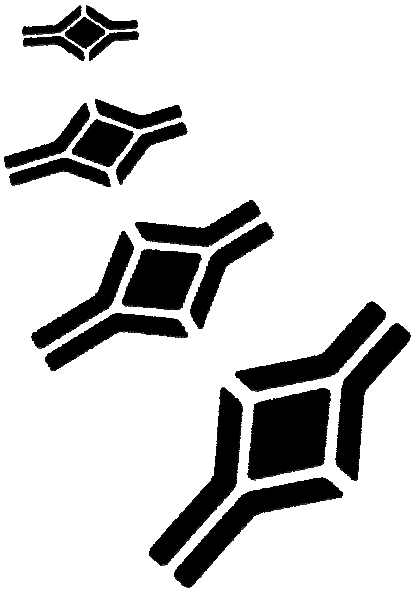

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