Human Resistin ELISA Kit
User Manual

UM-100301
Published 01 August 2004
Catalog# K1003-1

See List of Components for Storage Conditions
FOR RESEARCH USE ONLY

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I.   Introduction and Protocol Overview

Resistin, a product of the RSTN gene, is a peptide hormone belonging to the class of cysteine-rich secreted proteins which are termed the RELM family, and is also described as ADSF (Adipose Tissue-Specific Secretory Factor) and FIZZ3 (Found in Inflammatory Zone). Human resistin contains 108 amino acids as a pre-peptide. Its hydrophobic signal peptide is cleaved before it is secreted. Resistin circulates in human blood as a dimeric protein consisting of two 92 amino acid polypeptides, which are disulfide-linked via Cys26.

Much of the early investigations about the resistin molecule are based on the mouse model. Resistin produced and secreted primarily by adipocytes in mice, acts on skeletal muscle myocytes, hepatocytes and adipocytes themselves so that it reduces their sensitivity to insulin. Steppan et al. have suggested that resistin suppresses the ability of insulin to stimulate glucose uptake. Other studies have shown that mouse resistin increases during the differentiation of adipocytes, but it also seems to inhibit adipogenesis.

Compared to the mouse model, human adipogenic differentiation is likely to be associated with a down regulation of resistin gene expression. On the other hand, resistin was found to be expressed at high levels in human monocytes, macrophages, and bone marrow.
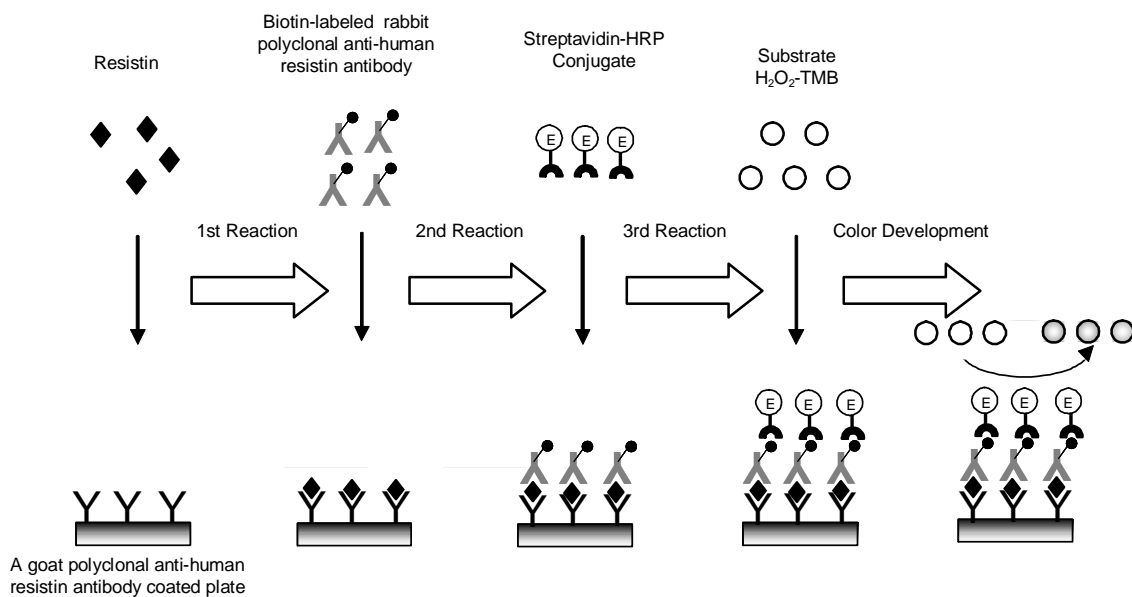
Recent investigations have shown that human resistin is correlated with metabolic syndrome and obesity-related disorders. Malo et al. have reported that resistin levels are positively associated with waist circumference, tumor necrosis factor-α, and insulin resistance assessed by the homeostasis model and inversely correlated with total cholesterol, HDL cholesterol, and LDL cholesterol. Moreover, Sadhasiv et al. found a positive correlation of SAT (subcutaneous adipose tissue) resistin mRNA expression with serum resistin, BMI and insulin resistance (HOMA index).

Based on the above reports human resistin might be an important marker that acts as the link between obesity and insulin resistance. Resistin can play a role also in inflammation processes and in atherosclerosis.

The B-Bridge **Human Resistin ELISA Kit** is designed to measure the concentration of human resistin (homodimeric) in human serum, plasma (EDTA, citrate, heparin), or tissue culture medium.

The principle of the assay is shown in Figure 1. Standards or samples are incubated in microtiter wells coated with a goat polyclonal anti-human resistin antibody. The wells are washed, and a biotin-labeled rabbit polyclonal anti-human resistin antibody is added and incubated with the captured resistin. After a thorough wash, streptavidin-horseradish peroxidase conjugate is added. Following subsequent incubation and washing, the bound conjugate is allowed to react with the substrate H_2O_2-tetramethylbenzidine (TMB). The reaction is quenched by addition of acidic solution and absorbance of the resulting product is measured at 450 nm. The absorbance is proportional to the concentration of resistin. A standard curve is constructed by plotting absorbance values versus resistin concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

Figure 1. Assay Principle

II.   List of Components

- **Store all components at 2-8℃.   DO NOT FREEZE.**

1	**10X WASH SOLUTION**	1 Bottle (100 ml)
2	**1X SAMPLE DILUENT**	1 Bottle (20 ml)
3	**PRIMARY ANTIBODY-COATED PLATE** One plate holds 12x8-well strips (96 wells), with adsorbed Goat Polyclonal Anti-Human Resistin Antibody. Plate is provided in a resealable foil pouch with desiccant.	1 Plate
4	**HUMAN RESISTIN MASTER STANDARD** Lyophilized	1 Vial
5	**QUALITY CONTROL** High and Low, lyophilized	2 Vials
6	**BIOTINYLATED SECONDARY ANTIBODY SOLUTION** Rabbit Polyclonal Anti-Human Resistin Antibody, Biotin Labeled	1 Bottle (13 ml)
7	**CONJUGATE SOLUTION** Streptavidin-Horseradish Peroxidase Conjugate	1 Bottle (13 ml)
8	**SUBSTRATE SOLUTION** H_2O_2 Tetramethylbenzidine	1 Bottle (13 ml)
9	**STOP SOLUTION** (0.2M H_2SO_4)	1 Bottle (13 ml)

MSDS forms are available on our website—please visit www.b-bridge.com

III.   Additional Materials Required

The following materials are required, but not supplied:

- Graduated cylinder

- Micropipettor(s) and disposable pipette tips

- Null strips for 96-well plate

- 96-well plate washer

- Paper towels or absorbent paper

- Plate reader capable of measuring absorbance at a wavelength of 450 nm

- Orbital microplate shaker capable of approximately 300 rpm

- Tubes for sample dilutions

- Deionized water

IV.   Reagent Preparation and Storage

Allow all the reagents to equilibrate at room temperature (25℃) prior to the start of the reagent preparation.

1. 1X Wash Solution
    Prepare 1X Wash Solution by mixing 100 ml of 10X Wash Solution with 900 ml of deionized water or equivalent.   After preparation, store 1X Wash Solution at 2-8℃.   The diluted Wash Solution is stable for 1 month at 2-8℃.

2. Human Resistin Standard Solution
    Reconstitute the Resistin Master Standard according to the Certificate of Analysis. Let the lyophilized standard dissolve in 1X Sample Diluent for at least 15 minutes prior to serial dilutions for the assay.   Prepare each Resistin Standard (20 ng/ml, 10 ng/ml, 5 ng/ml, 2 ng/ml, and 1 ng/ml) by serially diluting the stock Resistin Master Standard Solution (50 ng/ml) with 1X Sample Diluent as shown in the table.   Use the reconstituted Resistin Standard (50 ng/ml) and 1X Sample Diluent for the 50.0 ng/ml and 0 ng/ml standard solutions, respectively.

    Table 1: Serial Dilution of Resistin Standards

Master Standard Vol.	1X Diluent	Concentration
Master Std. Stock	-	50 ng/ml
500 µl of Master Std. Stock	750 µl	20 ng/ml
500 µl of 20 ng/ml Std.	500 µl	10 ng/ml
500 µl of 10 ng/ml Std.	500 µl	5 ng/ml
500 µl of 5 ng/ml Std.	750 µl	2 ng/ml
500 µl of 2 ng/ml Std.	500 µl	1 ng/ml

    Further dilute the serial diluted Standard Solutions 1:3 prior to use by mixing 100 µl of Standard Solutions with 200 µl of Sample Diluent for duplicates.   Do not store the diluted standard solutions.

    Reconstituted and stock Resistin Standard Solutions (50 – 1 ng/ml) should be aliquoted and frozen at -20℃ for 3 months. Do not store the dilut ed (1:3) standard solutions.

3. Quality Controls
    Reconstitute the Quality Controls with 350 µl Sample Diluent, mix and let sit for 30 minutes at room temperature. Further dilute the reconstituted Quality Controls 1:3 with Sample Diluent prior to use (50 µl control + 100 µl Sample Diluent for singlets or 100 µl Quality Control + 200 µl Sample Diluent for duplicates).   Reconstituted Quality Controls are stable until the expiration date at 2-8℃.   Do not store the 1:3 diluted Qualit y Controls.

    Refer to the Certificate of Analysis for Quality Control concentrations. The Quality Controls serve to confirm that the kit works in accordance with Certificate of Analysis and the ELISA test was carried out properly.

V.   Sample Preparation

Allow all the reagents to equilibrate at room temperature (25°C) prior to the start of the sample preparation.

Samples should be assayed immediately after collection or stored at -20°C or -70°C for long term. Thoroughly mix thawed samples prior to assaying.

Aliquot samples to avoid repeat freeze thaw cycles. Avoid using hemolyzed or lipemic samples.

Serum and plasma samples:

1.   For Assaying Samples in singlets:
      Mix 50 µl of samples with 100 µl of 1X Sample Diluent.

2.   For Assaying Samples in duplicates:
      Mix 100 µl of samples with 200 µl of 1X Sample Diluent.

Do not store 1:3 diluted samples.

Tissue culture supernatant:

Quantity of protein in cell culture supernatant may vary by cell line (e.g. HIB 1B, LS14 etc.), we recommend running several dilutions to determine optimal dilution for the assay. Undiluted culture supernatant can also be assayed.

Select a few samples to determine optimal dilution for assaying.
1.   Undiluted tissue culture supernatants

2.   Dilute 1:3 with 1X Sample Diluent:
      50µl of sample + 100 µl of Sample Diluent

3.   Dilute 1:6 with 1X Sample Diluent
      25µl of sample + 125 µl of Sample Diluent

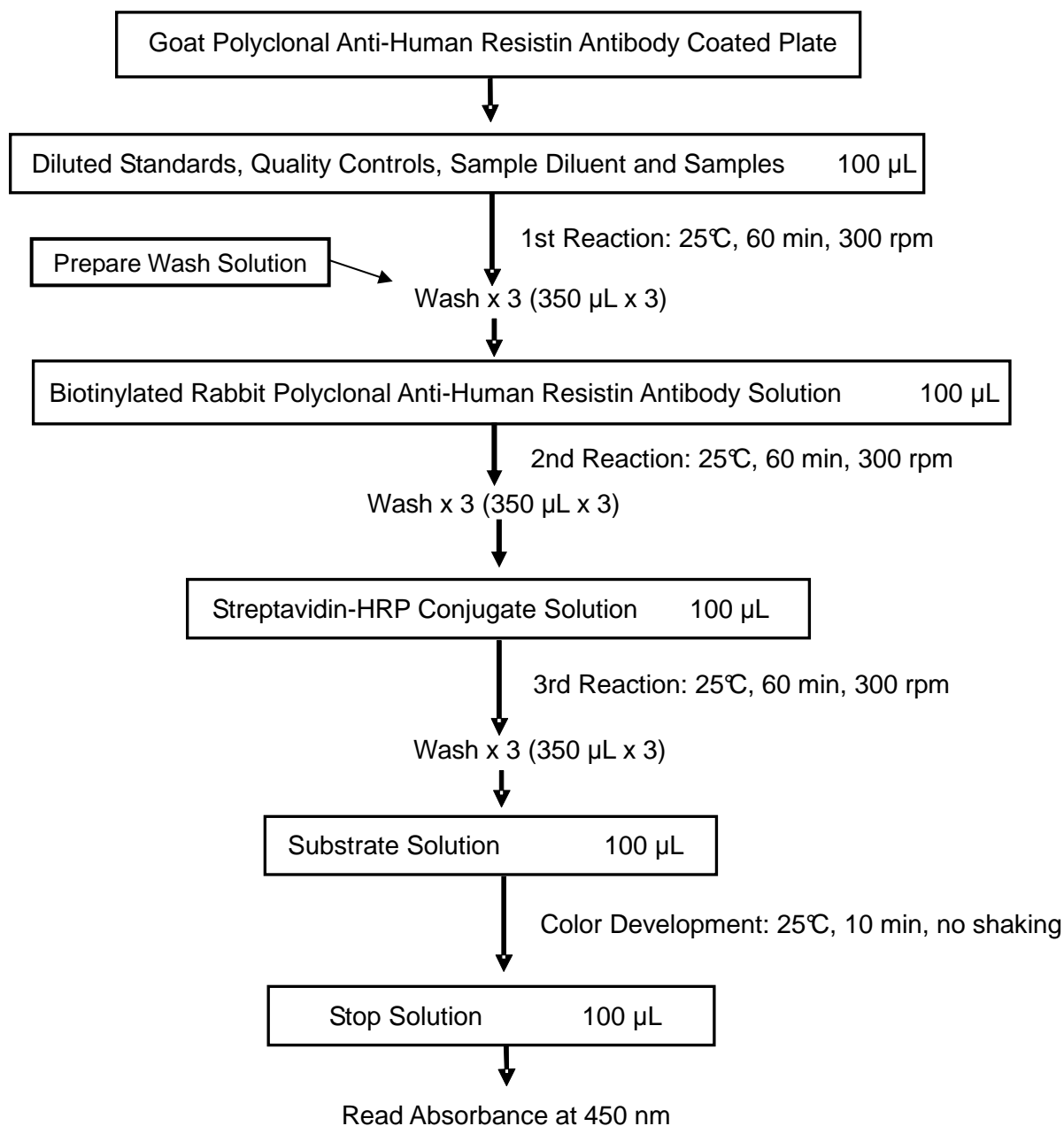Choose suitable dilution and repeat the assay with all samples.

VI.   Human Resistin ELISA Protocol

Note: Allow all reagents to equilibrate at room temperature (25℃) prior to the start of the assay and prepare 1X Wash Solution, Quality Controls, Resistin Standards, and samples as described in the previous sections.

1. Remove Primary Antibody-Coated Plate from its foil pouch. Remove any unneeded strips from the plate frame, reseal them in the foil pouch, and return the foil pouch to 2-8℃.   If a 96-well plate washer is used, the plate frame should be completely filled with wells by adding as many null strips as necessary. Identify well position(s) for each sample on a data sheet or plate map.

2. Add 100 µl of diluted Standards, Quality Controls, Sample Diluent (blank) and samples to the appropriate number of antibody-coated wells. Every plate must include the standard series to properly correlate the sample readings.

3. Cover plate(s) securely and incubate at room temperature (25℃) for 60 minutes on an orbital microplate shaker at ca. 300 rpm.

4. After incubation, wash the plate(s) 3 times with Wash Solution as follows:
    a. Completely aspirate the liquid from the wells using a plate washer.
    b. Fill each well with 1X Wash Solution (~350 µl/well) and immediately aspirate. Avoid overflow.
    c. Repeat Step 4b two more times for a total of three washes.
    d. Invert the plate(s) and gently tap on a clean absorbent towel.

5. Dispense 100 µl of the Biotinylated Secondary Antibody Solution into each well.

6. Cover plate(s) securely and incubate at room temperature (25℃) for 60 minutes on an orbital microplate shaker at ca. 300 rpm.

7. Repeat the wash procedure described in step 4.

8. Dispense 100 µl of Conjugate Solution into each well.

9. Cover plate(s) securely and incubate at room temperature (25℃) for 60 minutes on an orbital microplate shaker at ca. 300 rpm.

10. Repeat the wash procedure described in step 4.

11. Dispense 100 µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight.   Covering the plate with aluminum foil is recommended.

12. Cover plate(s) securely and incubate at room temperature (25℃) for 10 minutes with no shaking. The incubation time may be extended up to 20 minutes, if the reaction temperature is below 20℃.

13. Dispense 100 µl of Stop Solution into each well.   The plate should be read immediately.

14. Read the plate at 450 nm using a plate reader with reference wavelength 630 nm. Subtract the readings at 630 nm from the readings at 450 nm.

Note: If some samples and standards 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 concentrations of off scale standards and samples.
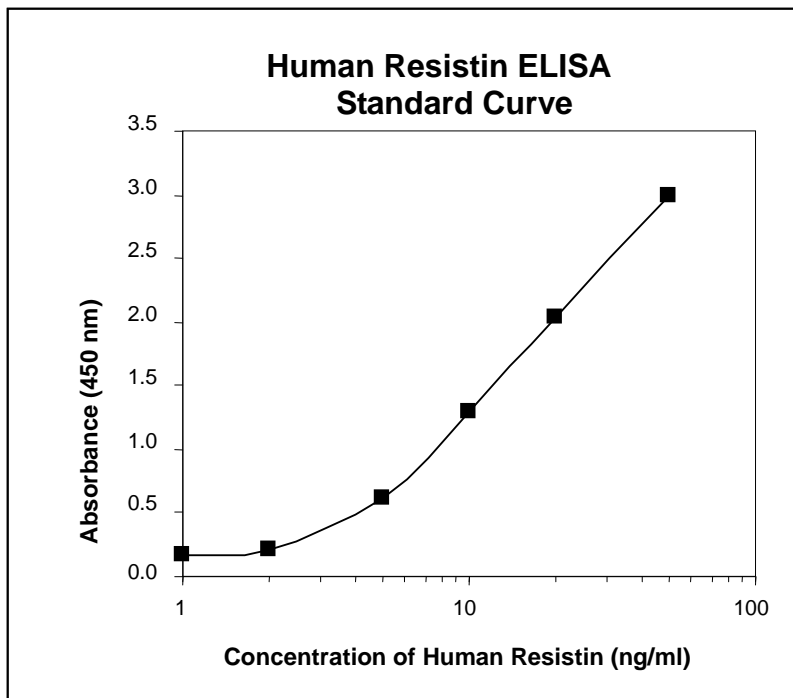
Figure 2.   Flow Chart of Assay Procedure

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┌──────────────────────────────────────────────────────────────────┐
│   Goat Polyclonal Anti-Human Resistin Antibody Coated Plate        │
└──────────────────────────────────────────────────────────────────┘
                                  ↓
┌──────────────────────────────────────────────────────────────────┐
│ Diluted Standards, Quality Controls, Sample Diluent and Samples  100 µL │
└──────────────────────────────────────────────────────────────────┘
                                  │ 1st Reaction: 25℃, 60 min, 300 rpm
┌─────────────────────────┐       │
│  Prepare Wash Solution   │─────→ │
└─────────────────────────┘   Wash x 3 (350 µL x 3)
                                  ↓
┌──────────────────────────────────────────────────────────────────┐
│ Biotinylated Rabbit Polyclonal Anti-Human Resistin Antibody Solution   100 µL │
└──────────────────────────────────────────────────────────────────┘
                                  │ 2nd Reaction: 25℃, 60 min, 300 rpm
                          Wash x 3 (350 µL x 3)
                                  ↓
┌──────────────────────────────────────────────────────────────────┐
│  Streptavidin-HRP Conjugate Solution       100 µL                  │
└──────────────────────────────────────────────────────────────────┘
                                  │ 3rd Reaction: 25℃, 60 min, 300 rpm
                          Wash x 3 (350 µL x 3)
                                  ↓
┌──────────────────────────────────────────────────────────────────┐
│  Substrate Solution          100 µL                                │
└──────────────────────────────────────────────────────────────────┘
                                  │ Color Development: 25℃, 10 min, no shaking
                                  ↓
┌──────────────────────────────────────────────────────────────────┐
│  Stop Solution          100 µL                                     │
└──────────────────────────────────────────────────────────────────┘
                                  ↓
                     Read Absorbance at 450 nm
```

VII. Calculation of Results

1. Subtract the mean absorbance value of the 0 ng/ml blank from each mean absorbance value of the standard series and samples tested (Net Absorbance).

2. Plot the log of known concentrations of each standard and the calculated Net Absorbances on the X-axis and Y-axis, respectively. Fit an appropriate regression curve to the plotted points.

3. Determine the resistin concentrations of the samples by interpolation of the regression curve formula.

Figure 3.   Typical Standard Curve

VIII.   Troubleshooting Guide

1.   Lack of signal or 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 25°C.
- Plate reader did not perform well.

2.   High signal and background in all wells
   Possible explanations:

- Improper or inadequate washing; be certain that all wash volumes and repetitions were correct.
- Decrease color development time.
- The plate should be read within 5 minutes of stopping the color development

3.   High background in sample wells only
   Possible explanations:

- Sample concentration was too high.

4.   Weak signal in sample wells only
   Possible explanations:

- Sample concentration was too low.

IX.   References

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