

B-Bridge International, Inc.



Rat Resistin ELISA Kit User Manual

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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 is termed the RELM family, and is also described as ADSF (Adipose Tissue-Specific Secretory Factor) and FIZZ3 (Found in Inflammatory Zone). Rat resistin contains 114 amino acids as a prepeptide, and its hydrophobic signal peptide is cleaved before secretion. Resistin circulates in rat blood as a dimeric protein consisting of two 96 amino acid polypeptides, which are disulfide-linked.

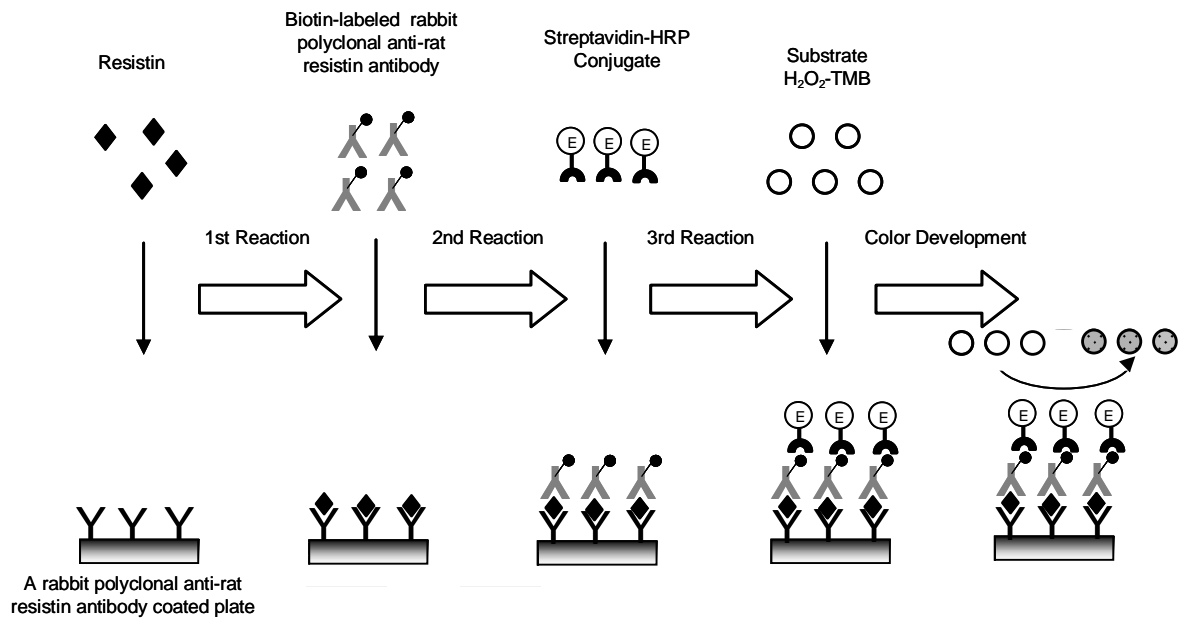
Resistin may be an important link between obesity and insulin resistance. Mouse resistin, specifically produced and secreted by adipocyte, acts on skeletal muscle myocytes, hepatocytes and adipocytes to reduce their sensitivity to insulin. Steppan et al. have suggested that resistin suppresses the ability of insulin to stimulate glucose uptake. They have also suggested that resistin is present at elevated levels in the blood of obese mice, and is down regulated by fasting and antidiabetic drugs (TZDs). On the other hand, several studies demonstrated reduced resistin expression in adipose tissue of obese mice and increased levels in leptin deficient *ob/ob* mice and Zucker diabetic fatty rats in response to TZDs.

Other studies have shown that mouse resistin increases during the differentiation of adipocytes, but it also seems to inhibit adipogenesis. Nevertheless, the human adipogenic differentiation is likely to be associated with a down regulation of resistin gene expression.

The B-Bridge **Rat Resistin ELISA Kit** is designed to measure the concentration of rat resistin in rat serum 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 rabbit polyclonal anti-rat resistin antibody. The wells are washed and biotin-labelled rabbit polyclonal anti-rat resistin antibody is added and incubated with the captured resistin. After a thorough wash, streptavidin-horseradish peroxidase (HRP) 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 correlates to the concentration of resistin. A standard curve is constructed by plotting absorbance values versus known resistin standard concentrations and concentrations of unknown samples are determined by interpolation on this curve.

Figure 1. Assay Principle



II. List of Components

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

| | | |
|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| 1 | 10X WASH SOLUTION | 1 Bottle (100 ml) |
| 2 | 1X SAMPLE DILUENT | 2 Bottle (10 ml) |
| 3 | PRIMARY ANTIBODY-COATED PLATE One plate holds 12x8-well strips (96 wells), with adsorbed Rabbit Polyclonal Anti-Rat Resistin Antibody. Plate is provided in a resealable foil pouch with desiccant. | 1 Plate |
| 4 | RAT RESISTIN MASTER STANDARD 20 ng, lyophilized | 1 Vial |
| 5 | QUALITY CONTROL High and Low, lyophilized | 2 Vial |
| 6 | BIOTINYLATED SECONDARY ANTIBODY SOLUTION Rabbit Polyclonal Anti-Rat Resistin Antibody, Biotin Labeled | 1 Bottle (13 ml) |
| 7 | CONJUGATE SOLUTION Streptavidin- Horseradish Peroxidase Conjugate | 1 Bottle (13 ml) |
| 8 | SUBSTRATE H ₂ O ₂ Tetramethylbenzidine (TMB) | 1 Bottle (13 ml) |
| 9 | STOP SOLUTION (0.2M H ₂ SO 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
- Well-closed containers such as microtubes (1.5 ml or more in capacity)
- Deionized water

IV. Reagent Preparation and Storage

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

1. 1X Wash Solution

Prepare 1X Wash Solution by mixing all of the 10X Wash Solution (100 ml) with 900 ml of deionized water. After preparation, store 1X Wash Solution at 2-8°C. The diluted Wash Solution is stable for 1 month at 2-8°C.

2. Rat Resistin Master Standard Solution

Reconstitute Resistin Standard with 1 ml Sample Diluent. The concentration of the reconstituted Resistin Standard is 20 ng/ml. Make serial dilutions of the standard as follows:

Table 1: Serial Dilution of Resistin Standards

| Master Standard Vol. | 1X Diluent | Concentration |
|--------------------------------|------------|---------------|
| Master Std. | - | 20 ng/ml |
| 500 µl of 20 ng/ml Master 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. | 500 µl | 2.5 ng/ml |
| 500 µl of 2.5 ng/ml Std. | 750 µl | 1 ng/ml |
| 500 µl of 1 ng/ml Std. | 500 µl | 0.5 ng/ml |
| 500 µl of 0.5 ng/ml Std. | 500 µl | 0.25 ng/ml |

The standards are stable until the expiration date at -20°C.

3. Quality Controls

The high and low Quality Controls should be reconstituted with 350 µl of Sample Diluent. After reconstitution, the controls are ready to use and require no further dilutions. Reconstituted Quality Controls are stored at -20°C and are stable until the expiration date.

Note: Do not mix reagents from different kits unless they have the same lot number.

V. Sample Preparation and Storage

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

1. For Assaying Samples in singlets
Mix 10 µl of samples with 190 µl of 1X Sample Diluent.
2. For Assaying Samples in duplicates
Mix 15 µl of samples with 285 µl of 1X Sample Diluent.

Note:

Serum samples are stable for 1 year when stored frozen at –20°C. Repeated freeze-thawing should be avoided.

Undiluted samples are stable for a week at 4°C. To avoid microbial contamination, add NaN₃ to a final concentration of 0.1% to the samples.

Diluted samples should not be stored.

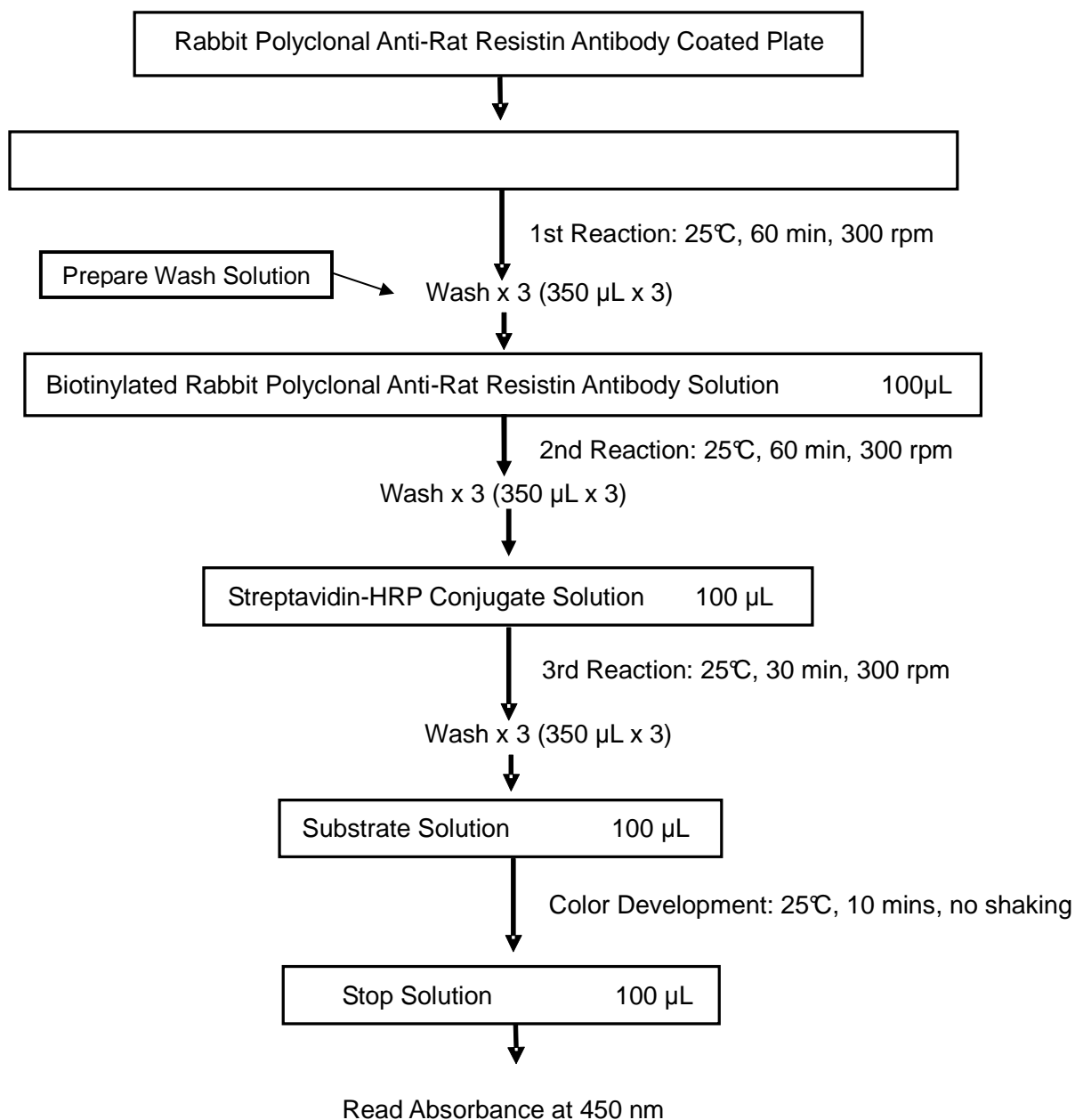
VI. Rat Resistin ELISA Protocol

Note: Allow all reagents to come to room temperature (25°C) 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°C. 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 (20, 10, 5, 2.5, 1, 0.5, 0.25 ng/ml), Quality Controls, Sample Diluent (blank), and samples to the appropriate number of antibody-coated wells. Every plate must include the standards to properly correlate the sample readings.
3. Cover plate(s) securely and incubate at room temperature (25°C) for 60 minutes on an orbital microplate shaker at ca. 300 rpm.
4. After incubation, thoroughly 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°C) 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 Streptavidin-HRP Conjugate Solution into each well.
9. Cover plate(s) securely and incubate at room temperature (25°C) for 30 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°C) for 10 minutes with no shaking. The incubation time may be extended up to 20 minutes, if the reaction temperature is below 20°C.
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.

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. The standard curve constructed using the values measured at 405 nm can be used to determine resistin concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Figure 2. Flow Chart of Assay Procedure



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 concentrations of each standard and the calculated Net Absorbance on the X-axis and Y-axis, respectively. Fit an appropriate regression curve to the points.
3. Determine the resistin concentrations of the samples by interpolation of the regression curve.
4. If the samples were diluted 1:20, the values for samples calculated from the calibration curve have to be multiplied by a dilution factor of 20 to obtain true concentration values.

Figure 3. Typical Standard Curve

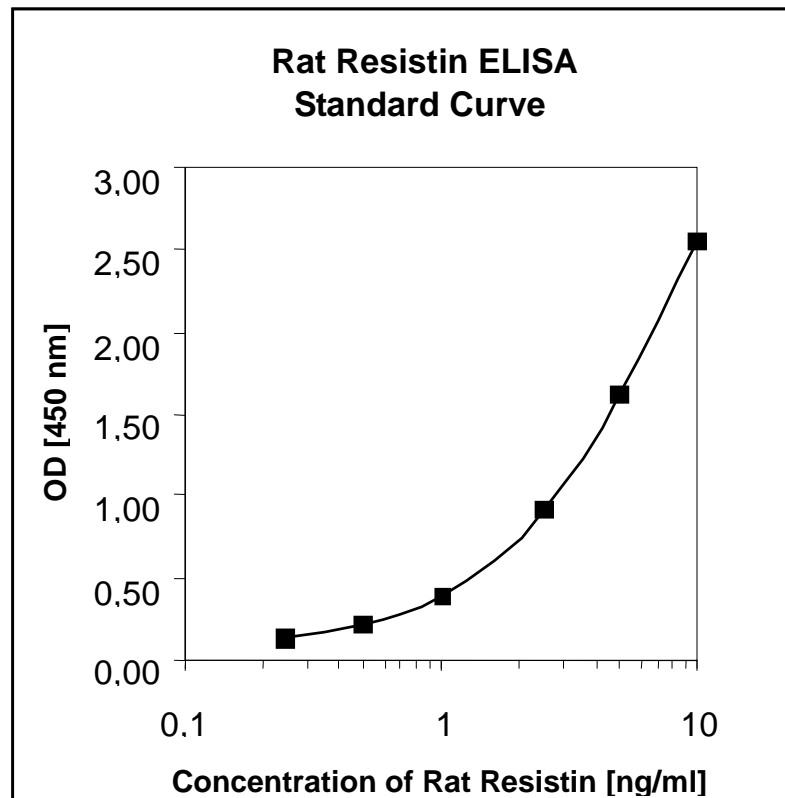


Figure 3: Standard Curve for rat resistin is plotted using the four-parameter function as a proportion of rat resistin concentration and absorbance at 450 nm.

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.

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