

**B-Bridge International, Inc.**



# Mouse 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 (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 hydrophobic 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.

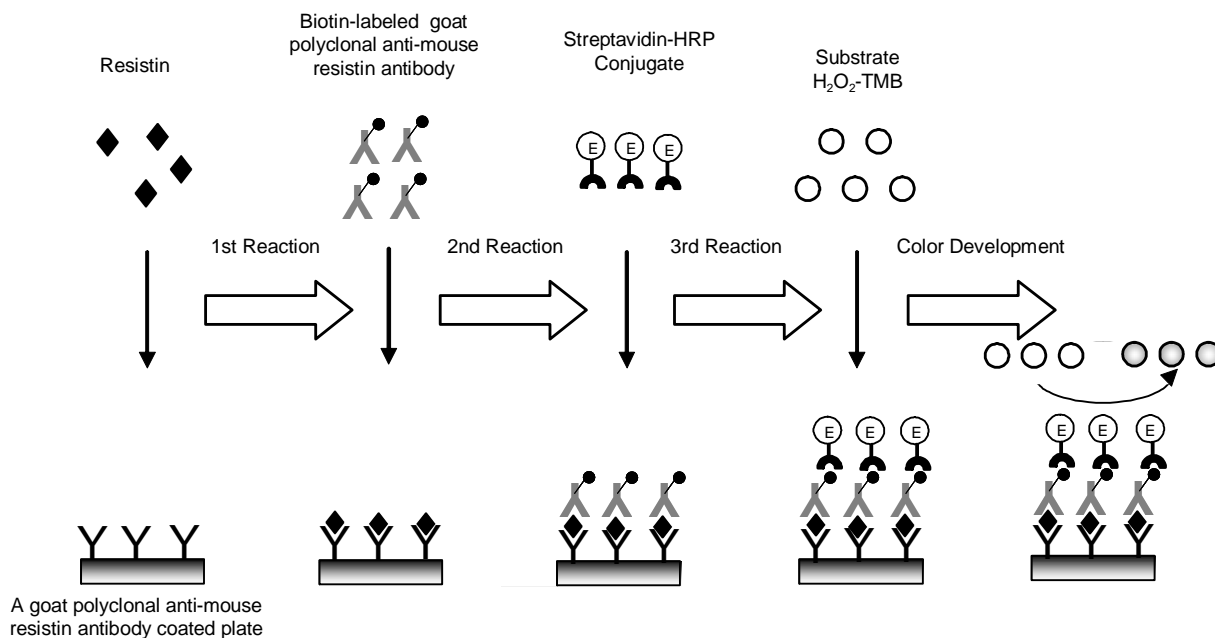
Resistin may be an important link between obesity and insulin resistance. Mouse resistin, specifically produced and secreted by adipocyte, acts on skeletal muscle myocytes, hepatocytes and adipocytes themselves so that it reduces their sensitivity to insulin. Steppan et al. have suggested that resistin 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 anti-diabetic drugs. Way et al., on the other hand, have found that resistin expression is severely suppressed in obesity and is stimulated by several anti-diabetic 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.

The B-Bridge Mouse Resistin ELISA Kit is designed to measure the concentration of mouse resistin in serum, plasma (EDTA or citrate), and 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-mouse resistin antibody. The wells are washed and biotin-labeled goat polyclonal anti-mouse 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 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.

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1	<b>10X WASH SOLUTION</b>	1 Bottle (100 ml)
2	<b>5X SAMPLE DILUENT</b>	1 Bottle (22 ml)
3	<b>PRIMARY ANTIBODY-COATED PLATE</b> One plate holds 12x8-well strips (96 wells), with adsorbed Goat Polyclonal Anti-Mouse Resistin Antibody. Plate is provided in a resealable foil pouch with desiccant.	1 Plate
4	<b>MOUSE RESISTIN STANDARDS</b> 1, 2, 5, 10, 20, 50, 100 ng/ml	7 Vials (50 µl each)
5	<b>QUALITY CONTROL</b> Lyophilized	1 Vial
6	<b>BIOTINYLATED SECONDARY ANTIBODY SOLUTION</b> Goat Polyclonal Anti-Mouse Resistin Antibody, Biotin Labeled	1 Bottle (13 ml)
7	<b>CONJUGATE SOLUTION</b> Streptavidin- Horseradish Peroxidase Conjugate	1 Bottle (13 ml)
8	<b>SUBSTRATE</b> H <sub>2</sub> O <sub>2</sub> Tetramethylbenzidine (TMB)	1 Bottle (13 ml)
9	<b>STOP SOLUTION</b> (0.2M H <sub>2</sub> SO <sub>4</sub> )	1 Bottle (13 ml)

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**MSDS forms are available on our website—please visit [www.b-bridge.com](http://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 450nm
- Orbital microplate shaker capable of approximately 300 rpm
- Tubes for diluting samples
- 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 Sample Diluent  
Prepare 1X Sample Diluent by mixing all of the 5X Sample Diluent (22 ml) with 88 ml of deionized water. The Diluent Buffer is stable for a month at 2-8°C.
2. 1X Wash Solution  
Prepare 1X Wash Solution by mixing all of the 10X Wash Solution (100 ml) with 900 ml of deionized water. The diluted Wash Solution is stable for 1 month at 2-8°C.
3. Mouse Resistin Master Standard Solution  
Prepare each Resistin Standard by diluting 10 µl of the stock standard solutions with 990 µl Sample Diluent for duplicates. Do not store the diluted standard solutions.
4. Quality Controls  
Reconstitute the lyophilized Quality Control by adding 50 µl of deionized water to the vial. Allow it to dissolve for at least 15 minutes and vortex thoroughly. The reconstituted control serum has to be used immediately or stored frozen.

Dilute the reconstituted Quality Controls 1:100 with Sample Diluent (10 µl with 990 µl Sample Diluent for duplicates).

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. Dilute serum and plasma samples 1:100 with Sample Diluent. Mix 10µl of samples with 990 µl of Sample Diluent for duplicates.

***Note: Serum samples should be stored frozen preferably at –80°C. Repeated thawing-freezing cycles should be avoided. Undiluted samples are stable at least a week at 2-8°C or one day at room temperature (25°C). Diluted samples have to be stored frozen.***

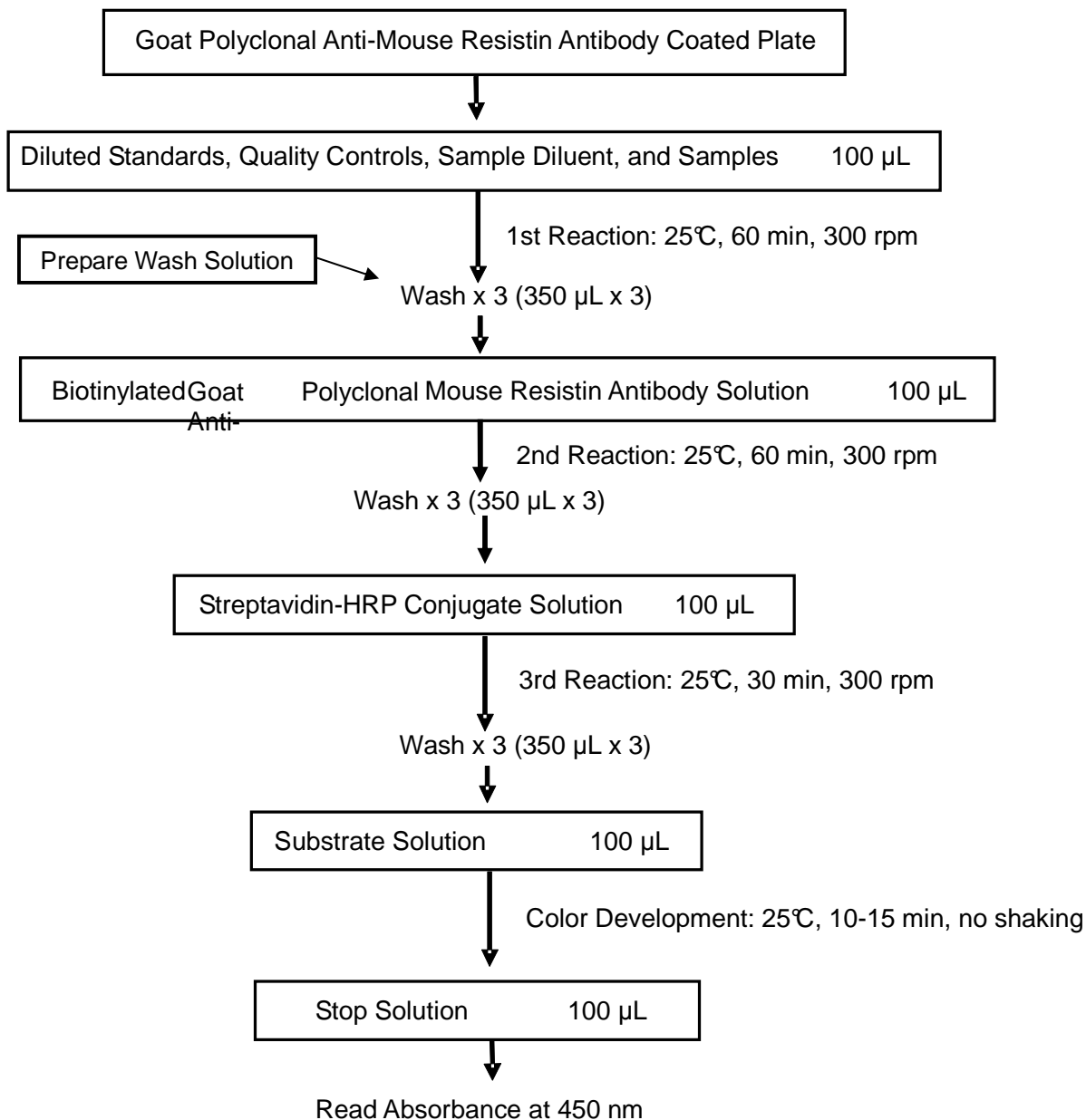
## VI. Mouse Resistin ELISA Protocol

**Note:** Allow all reagents to come to room temperature (25°C) prior to the start of the assay and prepare 1X Wash Solution, 1X Sample Diluent, 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, 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°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 to 15 minutes with no shaking.
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. If using a dual filter instrument, the recommended reference wavelength is 620-650 nm.

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

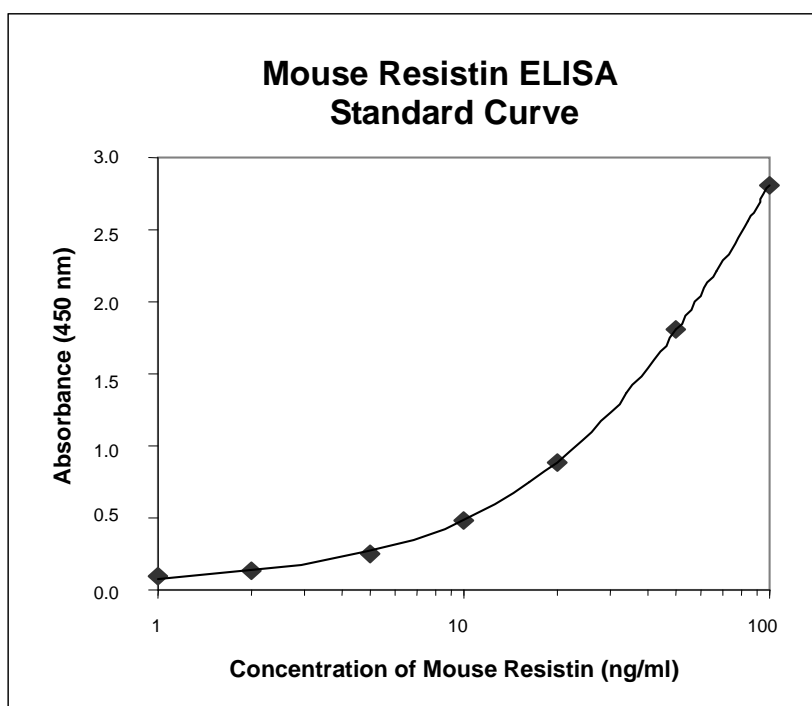
**Figure 2. Flow Chart of Assay Procedure**



## 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.

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.

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