Mouse Resistin Immunoassay

Catalog Number: EA800169

For the quantitative determination of mouse Resistin concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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BACKGROUND

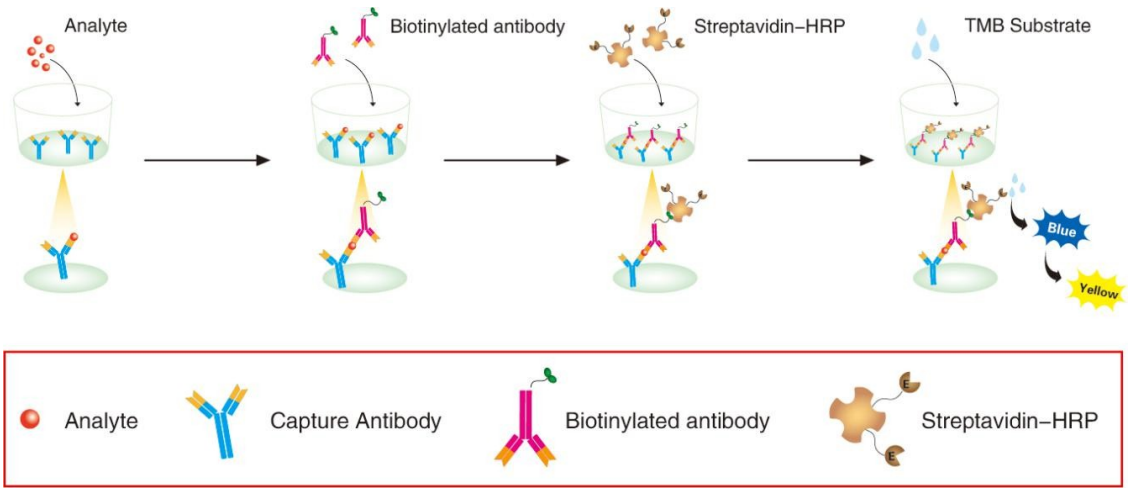
Resistin, also known as Found In Inflammatory Zone 3 (FIZZ3) or Adipocyte Secreted Factor (ADSF), is a member of a protein family known as the Resistin-like molecules (RELMs). It is perhaps best known for its potential as a link between obesity and the development of insulin resistance. The Resistin amino acid (aa) sequence contains a putative N-terminal signal sequence and a motif containing 11 cysteine residues, 10 of which are characteristic of the RELM family. The protein is thought to be secreted as a dimer and potentially exists in higher order molecular structures resulting from interactions between Resistin dimers or other members of the RELM family. A splice variant in the rat, lacking the signal sequence and localized predominantly to the nucleus, has also been described. A large 3' intron is the primary reason that the mouse genomic sequence is 3-fold larger than the corresponding human sequence . Mouse and human Resistin share only 53 percent identity at the aa level and exhibit differences in expression patterns.

Resistin acquired initial attention as a potential link between obesity and glucose regulation. Serum levels were shown to increase in diet-induced and genetic forms of obesity in mice (ob/ob and db/db) and decrease in response to insulin sensitizing drugs (TZDs). In vitro, Resistin expression by these cells is enhanced by treatment with several pro-inflammatory cytokines including IL-1β, TNF-α, IFN-γ, or IL-6. In addition, Resistin has been shown to activate endothelial cells in vitro, leading to the production of adhesion molecules, Endothelin-1, and chemokines

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Resistin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Resistin present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for Resistin is added to detect the captured Resistin protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



TECHNICAL HINTS AND LIMITATIONS

1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard -lyophilized, 2000 pg/vial upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard /Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

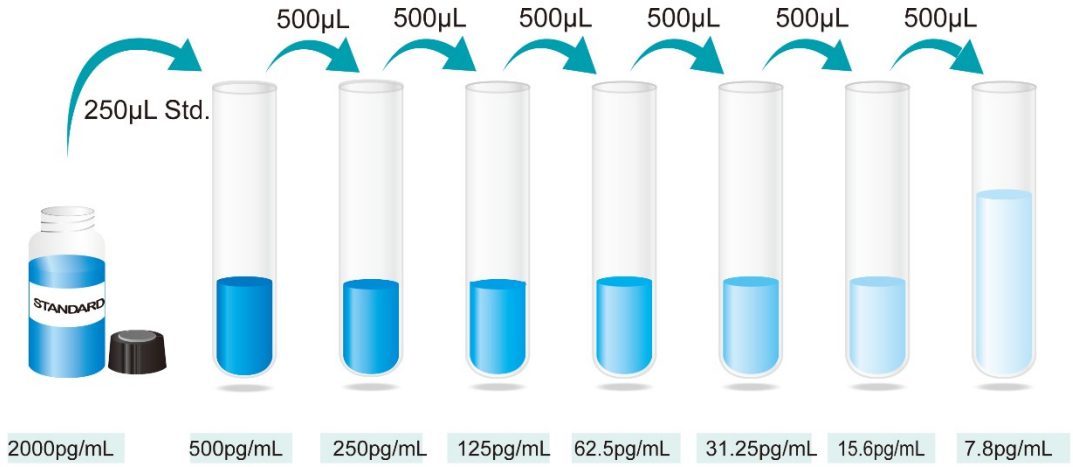
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: The normal mouse serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard/Specimen** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 750μL of Standard/Sample Diluent into the 500pg/mL tube, and add 250μL stock solution of 2000 pg/mL into it to get the high standard of 500pg/mL. Pipette 500μL of Standard/Sample Diluent into the remaining tubes. Use the high standard to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 500 pg/mL

standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of Resistin standard dilutions

***If you do not run out of re-melting standard, store it at -20°C. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-mouse Resistin antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

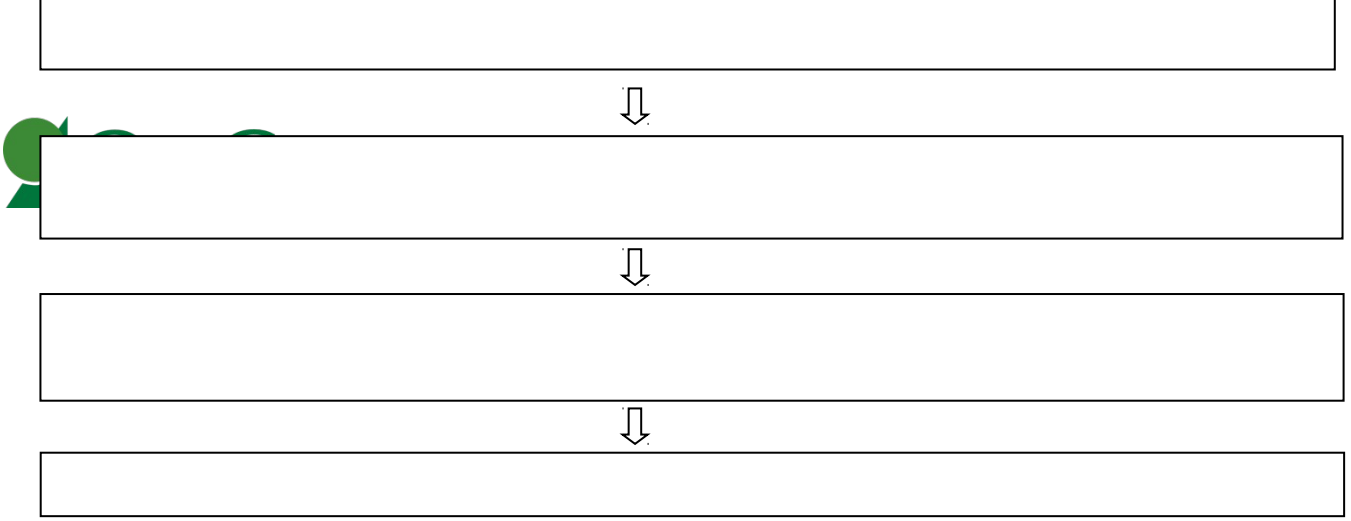
Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

Aspirate and wash 4 times

⇩



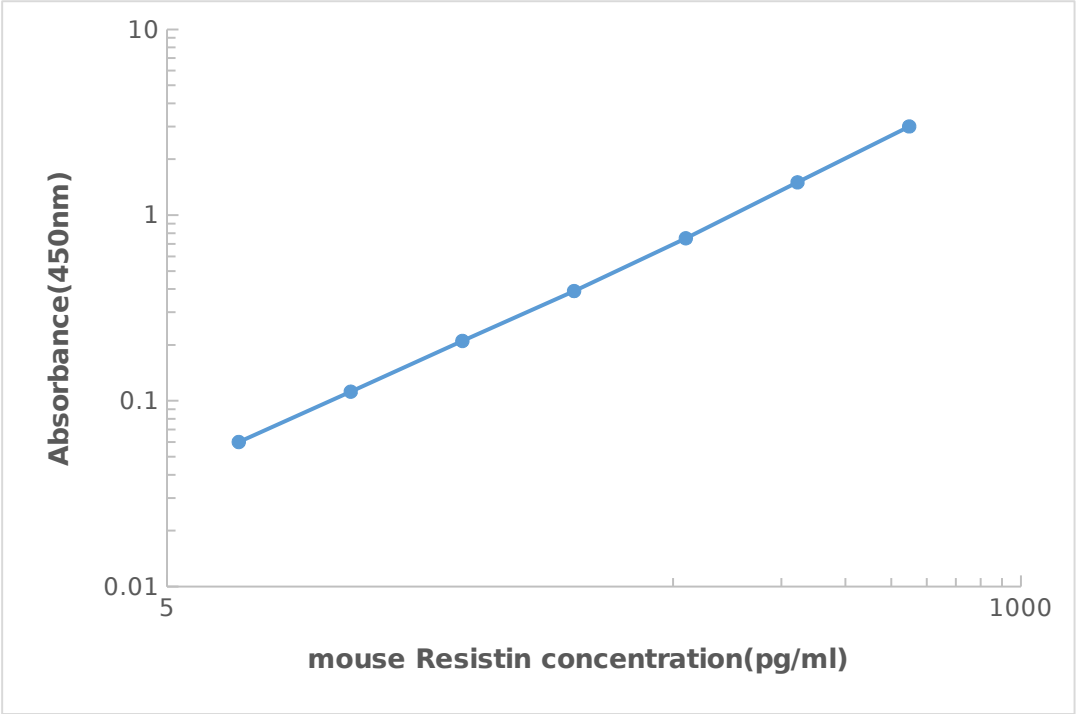
CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the Resistin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the Resistin ELISA

Standard(pg/	OD.	OD.	Average	Corrected

ml)				
0	0.018	0.023	0.020	-
7.81	0.215	0.219	0.217	0.197
15.6	0.248	0.253	0.250	0.230
31.25	0.369	0.376	0.372	0.352
62.5	0.586	0.598	0.592	0.571
125	0.951	0.971	0.961	0.941
250	1.548	1.580	1.564	1.544
500	2.523	2.575	2.549	2.529



Representative standard curve for Resistin ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 4 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant mouse Resistin. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and

assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant mouse	Recombinant rat	Recombinant human
Adiponectin/Acrp30		Resistin
IGF-I		
IGF-II		
IL-6		
Leptin		
TNF-α		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of Resistin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of Resistin in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	93	84－102
Cell culture supernatants	102	92－111

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of Resistin in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	95	101
	Range (%)	87－103	83－110
1:4	Average% of Expected	97	102
	Range (%)	89－108	95－109
1:8	Average% of Expected	95	107
	Range (%)	84-107	98-116
1:16	Average% of Expected	93	106
	Range (%)	85-101	95-117

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