Quantikine® ELISA

Mouse Resistin Immunoassay

Catalog Number MRSN00

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

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Mouse Resistin (or "resistance to insulin") is an 11 kD cysteine-rich polypeptide that is the charter member of a small family of inflammation-related factors (1-3). It is synthesized as a 114 amino acid (aa) precursor, with a 20 aa signal sequence and a 94 aa mature segment. It contains five intramolecular disulfide bonds and multiple β-turns (4). As noted, Resistin is a member of the RELM/FIZZ family that includes four molecules, each with 10 conserved cysteines. Two molecules, Resistin and RELMβ, have an additional 11th cysteine that may participate in a homodimeric disulfide linkage (2). Resistin itself is known to generate disulfide and nondisulfide-linked homodimers as well as multimers of varying size (2, 5). Notably, dimers are not necessary for bioactivity (4). Mature mouse Resistin shows 34%, 42%, and 40% aa homology to mouse RELMα, β, and γ, respectively (2, 3). Mouse to rat, there is 72% aa homology in the mature segment (1, 6). There is debate about the relationship between mouse and human Resistin. While the mature segments are 55% aa identical (1), the genes are markedly divergent in the promoter regions, leading some to suggest that the molecules have differing functions (7, 8). Recent evidence suggests, however, that this may not be the case (9). Rodent cells known to express Resistin include adipocytes (1, 10) intestinal epithelium and skeletal muscle cells (11), and, possibly, astrocytes (12). In humans, Resistin is found in syncytiotrophoblasts (13), monocytes/macrophages (14, 15) and adipocytes (16). The human adipocyte as a source of Resistin is a subject of debate. It is reported that mature adipocytes are absent of Resistin while preadipocytes are a source of Resistin (17, 18). Alternatively, mature human adipocytes are reported to produce Resistin (16), and the issue may be the timing of secretion and/or the fact that a disconnect exists between mRNA expression and protein secretion (19).

In mice, Resistin was originally named for its ability to resist (interfere with) insulin action (1). Resistin, given to normal mice, impaired glucose uptake without reducing insulin levels. Conversely, Resistin neutralization enhanced insulin-mediated glucose uptake by fat (1, 4). In rats, Resistin also interfered with insulin-stimulated glucose uptake by skeletal muscle, expanding the types of tissues impacted by Resistin (20). While these effects are accepted in rodents, uncertainty exists about the role of Resistin in humans. The structure of the human Resistin gene, and the principal source of cell expression (monocyte) in humans have led to suggestions that Resistin may have a different function (15, 17, 21). Recent studies now suggest two things. First, Resistin's relationship to insulin is functionally equivalent in both humans and mice. It is the principal source of production that is divergent (9, 19). Second, Resistin is proinflammatory, and shares a TNF-α like relationship with another adipocytokine, Adiponectin. Like TNF-α, Resistin activates endothelium. It induces the expression of ICAM-1 and VCAM-1 and promotes the secretion of MCP-1 and ET-1. Adiponectin, as it does with TNF-α, reverses these effects, providing a counterbalance to proinflammatory/atherogenic cytokines such as Resistin and TNF-α (22, 23).

The Quantikine Mouse Resistin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse Resistin in cell culture supernates, serum and plasma. It contains *E. coli*-expressed recombinant mouse Resistin and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse Resistin showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring mouse Resistin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse Resistin has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse Resistin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Resistin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse Resistin bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 10 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse Resistin Microplate	892559	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse Resistin.	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 °C.*
Mouse Resistin Standard	892561	Recombinant mouse Resistin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Mouse Resistin Control	892562	Recombinant mouse Resistin in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse Resistin Conjugate	892560	12 mL of a polyclonal antibody against mouse Resistin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5T	895175	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require at least a 30-fold dilution prior to assay. A suggested 30-fold dilution is 10 µL of sample + 290 µL of Calibrator Diluent RD5T.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

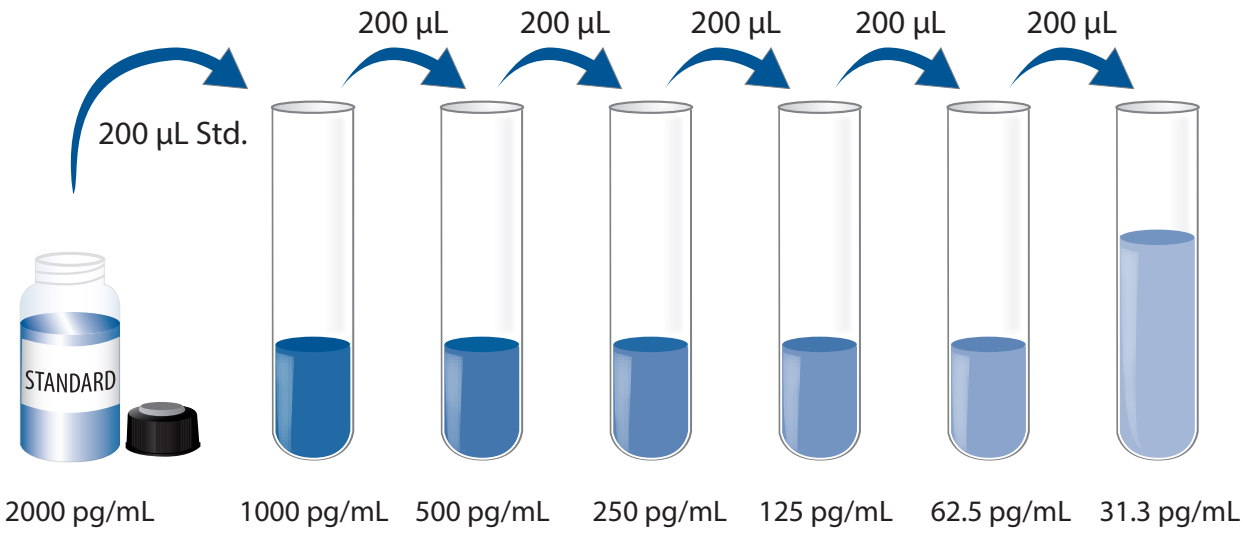
Mouse Resistin Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse Resistin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Resistin Standard with Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5T into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse Resistin Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5T serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, Control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, Control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse Resistin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

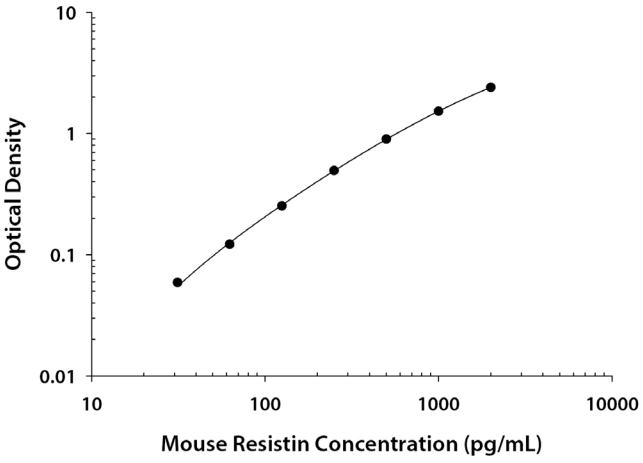
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

Create 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. The data may be linearized by plotting the log of the mouse 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.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.045 0.046	0.046	—
31.3	0.104 0.106	0.105	0.059
62.5	0.166 0.170	0.168	0.122
125	0.299 0.299	0.299	0.253
250	0.540 0.540	0.540	0.494
500	0.941 0.950	0.946	0.900
1000	1.561 1.580	1.571	1.525
2000	2.433 2.464	2.449	2.403

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty-one separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	41	41	41
Mean (pg/mL)	84	245	494	84	239	508
Standard deviation	3.4	7.2	13.3	6.1	16.4	34.9
CV (%)	4.0	2.9	2.7	7.3	6.9	6.9

RECOVERY

The recovery of mouse Resistin spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	99	85-108%
Serum* (n=6)	99	93-108%
EDTA plasma* (n=8)	97	87-107%
Heparin plasma* (n=8)	102	89-113%

*Samples were diluted 100-fold and then spiked prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse Resistin were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=7)	EDTA plasma* (n=8)	Heparin plasma* (n=7)
1:2	Average % of Expected	96	101	102	101
	Range (%)	94-98	97-107	95-108	99-103
1:4	Average % of Expected	98	101	102	104
	Range (%)	94-101	95-105	92-108	100-107
1:8	Average % of Expected	100	102	107	106
	Range (%)	97-104	96-108	100-110	101-111
1:16	Average % of Expected	100	106	112	108
	Range (%)	92-113	92-112	100-118	101-115

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of mouse Resistin ranged from 3-8 pg/mL. The mean MDD was 5 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse Resistin produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse Resistin in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=20)	46.1	17.6-84.0
EDTA plasma (n=20)	47.6	29.3-74.9
Heparin plasma (n=20)	49.0	27.6-72.9

Cell Culture Supernates - Mouse kidney, liver, lung, and heart tissues from one adult mouse (chopped in 1-2 mm pieces in 30 mL of medium) were cultured for 3 days in RPMI supplemented with 10% fetal calf serum and 50 µM β-mercaptoethanol. Mouse lung was additionally supplemented with 10 µg/mL of concanavalin A. Aliquots of the cell culture supernates were removed, assayed for levels of mouse Resistin, and measured 20.9 ng/mL, 1.83 ng/mL, 1.55 ng/mL, and 1.28 ng/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant mouse Resistin.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse Resistin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
Adiponectin/Acrp30
IGF-I
IGF-II
IL-6
Leptin
LIF
RELMα (aa 24-111)
TNF-α

Recombinant human:
RELMβ (aa 21-111)
Resistin

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