

Quantikine[®]

Human Resistin* Immunoassay

Catalog Number DRSN00
SRSN00
PDRSN00

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

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INTRODUCTION

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 (1). Other members of this family include RELM- α /FIZZ1 and RELM- γ , which are described in rodents but as yet have no identified human counterparts, and RELM- β /FIZZ2 (2, 3). 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 (1 - 3). 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 (4 - 7). A splice variant in the rat, lacking the signal sequence and localized predominantly to the nucleus, has also been described (8). A large 3' intron is the primary reason that the mouse genomic sequence is 3-fold larger than the corresponding human sequence (9). Mouse and human Resistin share only 53 percent identity at the aa level and exhibit differences in expression patterns (1, 9, 10). In mouse, expression appears primarily in adipose tissues (1). Although some human studies suggest Resistin is expressed by adipose tissues as well, the most significant source appears to be blood mononuclear cells (11 - 13). In humans, Resistin is also reported to be expressed by pre-adipocytes (14), placenta (15), pancreatic islets (16), and primary leukemia cells (10). A receptor for Resistin has not yet been described.

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) (1). In addition, function-blocking Resistin antibodies enhanced insulin actions while treatment with recombinant Resistin caused glucose intolerance and insulin resistance (1). Resistin knockout mice exhibit decreased fasting blood glucose levels as a result of reduced hepatic output (17). To establish a physiological role in humans, several studies have examined whether altered circulating Resistin levels are associated with type 2 diabetes, insulin resistance, and/or obesity. Although some demonstrate significant correlations (18 - 23), others report no correlation (23 - 28), suggesting that in humans fundamental questions remain regarding Resistin's role in these pathophysiological processes (29, 30). Resistin expression by human mononuclear cells could indicate a potential role in inflammation. *In vitro*, Resistin expression by these cells is enhanced by treatment with several pro-inflammatory cytokines including IL-1 β , TNF- α , IFN- γ , or IL-6 (31). In addition, Resistin has been shown to activate endothelial cells *in vitro*, leading to the production of adhesion molecules, Endothelin-1, and chemokines (32, 33).

The Quantikine Human Resistin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Resistin in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human Resistin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Resistin showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Human Resistin kit can be used to determine relative mass values for naturally occurring Resistin.

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 bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Resistin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Resistin bound in the initial step. The color development is stopped and the intensity of the color is measured.

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, 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.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Part #	Cat. # DRSN00	Cat. # SRSN00
Resistin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against Resistin.	892669	1 plate	6 plates
Resistin Conjugate - 21 mL/vial of monoclonal antibody against Resistin conjugated to horseradish peroxidase with preservatives.	892670	1 vial	6 vials
Resistin Standard - 100 ng/vial of recombinant human Resistin in a buffered protein base with preservatives; lyophilized.	892671	3 vials	18 vials
Assay Diluent RD1-19 - 11 mL/vial of a buffered protein base with preservatives.	895467	1 vial	6 vials
Calibrator Diluent RD5K - 21 mL/vial of a buffered protein base with preservatives.	895119	1 vial	6 vials
Wash Buffer Concentrate - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
Stop Solution - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
Plate Covers - Adhesive strips.	—	4 strips	24 strips

DRSN00 contains sufficient materials to run an ELISA on one 96 well plate.

SRSN00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDRSN00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1-19	
	Calibrator Diluent RD5K	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	Use within 4 hours and discard after use. Prepare fresh for each assay.
	Standard	
Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*	

*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.
- Test tubes for standard and sample dilution.
- Human Resistin Controls (optional; available from R&D Systems).

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at approximately 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}$ 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 a 5-fold dilution. A suggested 5-fold dilution is 60 μL sample + 240 μL Calibrator Diluent RD5K.

REAGENT PREPARATION

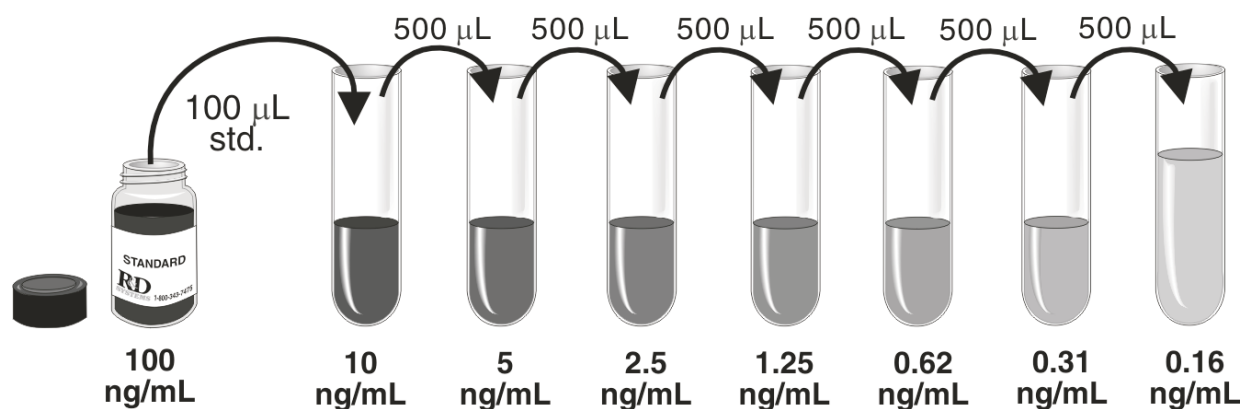
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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. 200 μL of the resultant mixture is required per well.

Resistin Standard - Reconstitute the Resistin Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μL of Calibrator Diluent RD5K into the 10 ng/mL tube. Pipette 500 μL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 ng/mL). **Prepare fresh for each assay. Use within 4 hours and discard after use.**



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, 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 100 μL of Assay Diluent RD1-19 to each well.
4. Add 100 μL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 μL of Resistin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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.

*Serum/plasma samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Add 100 μ L Assay Diluent RD1-19 to each well.



3. Add 100 μ L Standard, control or sample* to each well. Incubate 2 hours at RT.



4. Aspirate and wash 4 times.



5. Add 200 μ L Conjugate to each well. Incubate 2 hours at RT.



6. Aspirate and wash 4 times.



7. Add 200 μ L Substrate Solution to each well. Incubate 30 minutes. **Protect from light.**



8. Add 50 μ L Stop Solution to each well. Read at 450 nm within 30 minutes.
 λ correction 540 or 570 nm

*Serum/plasma samples 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.

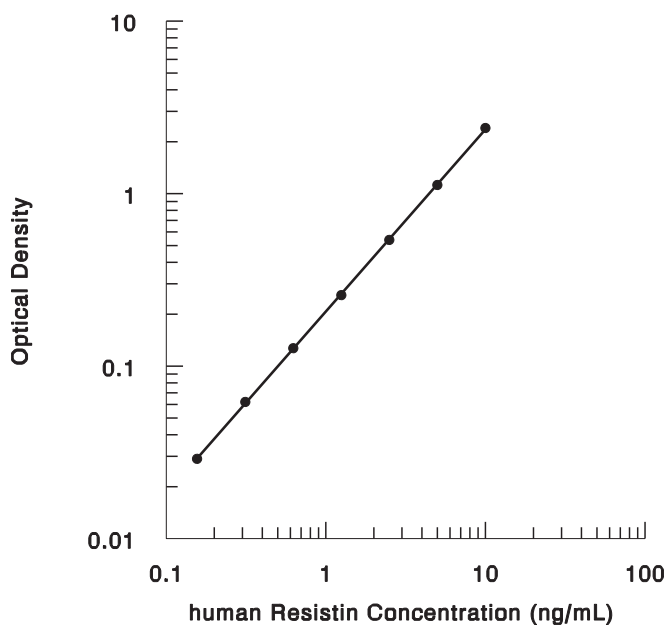
Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

To determine the Resistin concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding Resistin concentration.

If the 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.



ng/mL	O.D.	Average	Corrected
0	0.017 0.018 0.046	0.018	—
0.16	0.048 0.079	0.047	0.029
0.31	0.080 0.142	0.080	0.062
0.62	0.147 0.275	0.145	0.127
1.25	0.277 0.554	0.276	0.258
2.5	0.559 1.114	0.557	0.539
5	1.167 2.398	1.141	1.123
10	2.444	2.421	2.403

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.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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 separate assays to assess inter-assay precision.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.60	2.26	4.72	0.61	2.28	4.76
Standard deviation	0.03	0.12	0.18	0.05	0.21	0.37
CV (%)	5.0	5.3	3.8	8.2	9.2	7.8

RECOVERY

The recovery of Resistin spiked to levels throughout the range of the assay in tissue culture media and was evaluated.

Sample	Average % Recovery	Range
Tissue culture media (n=4)	99	96 - 103%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of Resistin were serially diluted with Calibrator Diluent RD5K to produce samples with values within the dynamic range of the assay.

		Tissue culture media (n=4)	Serum (n=5)	Heparin plasma (n=5)	EDTA plasma (n=5)
1:2	Average % of Expected	99	102	99	100
	Range (%)	95 - 102	99 - 104	95 - 107	99 - 102
1:4	Average % of Expected	98	100	99	100
	Range (%)	94 - 101	93 - 105	95 - 106	99 - 103
1:8	Average % of Expected	98	101	98	101
	Range (%)	95 - 101	89 - 110	94 - 104	99 - 105
1:16	Average % of Expected	93	96	95	97
	Range (%)	88 - 96	87 - 103	90 - 102	94 - 103

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of Resistin ranged from 0.010 - 0.055 ng/mL. The mean MDD was 0.026 ng/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 NS0-expressed recombinant human Resistin produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of Resistin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=45)	13.8	6.39 - 26.4	4.64
EDTA plasma (n=45)	11.2	5.38 - 24.6	4.72
Heparin plasma (n=44)	11.9	5.73 - 24.5	4.09

Cell Culture Supernates - Human peripheral blood cells (1×10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural Resistin.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	ND	ND
Stimulated	0.26	ND

ND = Non-detectable

SPECIFICITY

This assay recognizes recombinant and natural human Resistin. The factors listed below were prepared at 100 ng/mL in Calibrator Diluent RD5K and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Resistin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Leptin
Leptin R
LIF
RELM- β

Recombinant mouse:

Leptin
Leptin R
LIF
RELM- α
Resistin

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PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
	A	B	C	D	E	F	G	H

NOTES