Quantikine™ ELISA

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

TABLE OF CONTENTS

SECTION PAGE

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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-1β, 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 this kit can be used to determine relative mass values for natural human Resistin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human 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 human 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, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DRSN00	CATALOG # SRSN00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Resistin Microplate	892669	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human Resistin Standard	892671	2 vials	12 vials	Recombinant human Resistin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Prepare fresh for each assay. Use within 4 hours and discard after use.
Human Resistin Conjugate	892670	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human Resistin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-19	895467	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5K	895119	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

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). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human Resistin Microplate	892669	50 plates
Human Resistin Standard	892671	50 vials
Human Resistin Conjugate	892670	50 vials
Assay Diluent RD1-19	895467	50 vials
Calibrator Diluent RD5K	895119	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Inserts	751117	2 booklets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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 dilution of standards and samples
- Human Resistin Controls (Optional; R&D Systems®, Catalog # QC237)

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. Refer to the SDS 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 and 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 30 minutes at room temperature before centrifugation for 15 minutes at 1000 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 15 minutes at approximately 1000 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 a 5-fold dilution. A suggested 5-fold dilution is 60 µL of sample + 240 µL of Calibrator Diluent RD5K.

REAGENT PREPARATION

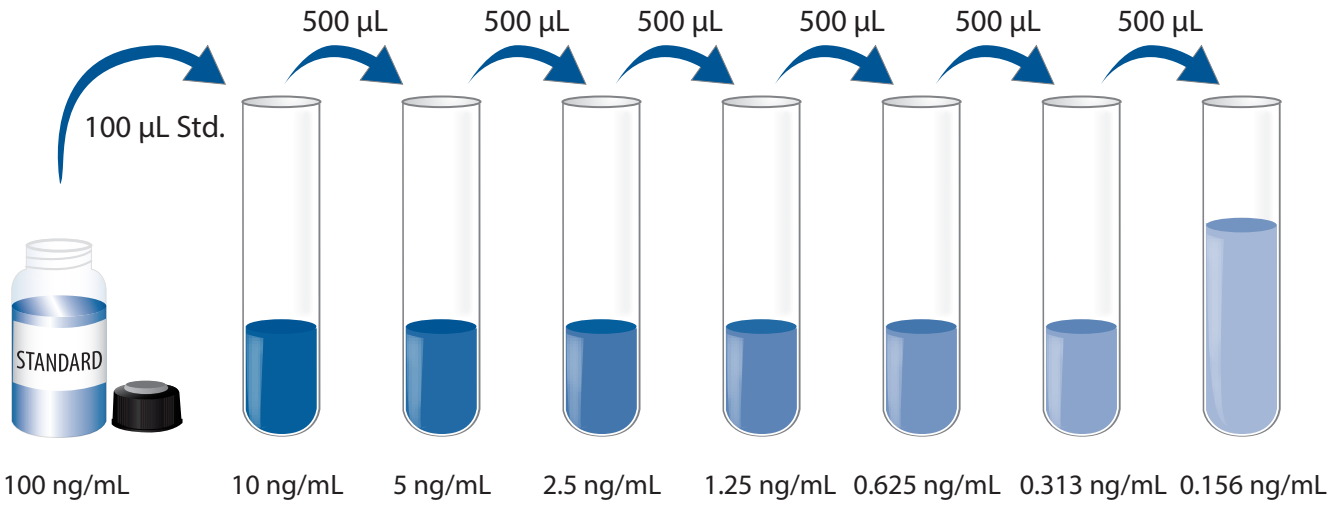
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate into 480 mL of 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.

Human Resistin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Resistin Standard with 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 standards, controls, and samples 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 Human 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

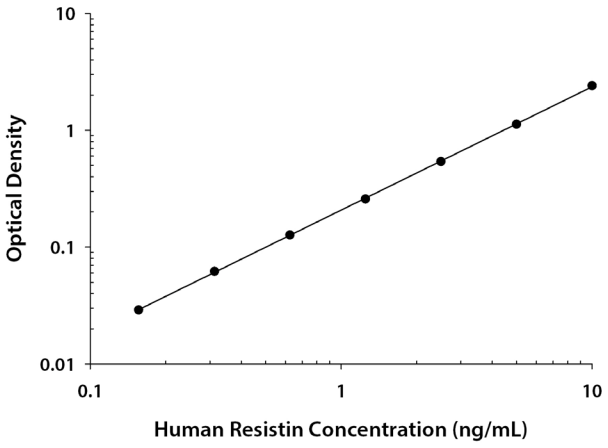
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the human Resistin concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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	0.018	—
0.156	0.046 0.048	0.047	0.029
0.313	0.079 0.080	0.080	0.062
0.625	0.142 0.147	0.145	0.127
1.25	0.275 0.277	0.276	0.258
2.5	0.554 0.559	0.557	0.539
5	1.114 1.167	1.141	1.123
10	2.398 2.444	2.421	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 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	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 human Resistin spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media	99	96-103%

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of human Resistin were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

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

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

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human 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 O.D. 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 human 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 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human Resistin.

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

ND-Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human Resistin.

The factors listed below were prepared at 100 ng/mL in calibrator diluent 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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