Ultrasensitive Resistin ELISA

For the quantitative determination of resistin in human serum and plasma samples.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 22-RESHUU-E01
Size: 96 wells
Version: 08.07.2021 Version 7- ALPCO 2.0

1. INTENDED USE

The Human Resistin ELISA is intended to be used for the quantitative determination of resistin in human serum or plasma samples. **For Research Use Only. Not for Use in Diagnostic Procedures.**

2. INTRODUCTION

Resistin, a cysteine-rich protein of 11.3 kDa (1), was first found in mice (2) and with RELMα, RELMß and RELMγ constitutes the protein family of resistin-like molecules (RELM).

In humans, resistin and RELMß (1), but no other proteins of the RELM family were found. The human form of resistin shows a homology of 53% to the murine protein (4). It has 11 cysteine residues, is synthesized as a propetide of 108 amino acids, and is secreted as a dimer, with a disulfide bridge of cysteine residues (22). Beside this intermolecular disulfide bridge, 5 additional intramolecular ones exist (5,6).

Appearance of multi- and oligomer formation was proved by size exclusion chromatography. Thereby it was shown that oligomer formation is SDS-insensitive but can be inhibited by β-mercaptoethanol and is therefore likely to be caused by disulfide bridges (1). Furthermore, the resistin structure seems to be concentration dependent, as circular dichroism analysis shows a concentration dependent shift of α-helical to ß-sheet structure (1).

Resistin expression was demonstrated in white adipose tissue (10), pituitary (11) and pancreatic islets (12) of mice as well as in brown adipose tissue of rats. In humans, resistin expression in adipocytes can be detected, but only at a very low level. However, in vitro resistin expression in nonadipocytes of fatty tissue was shown (13). Human resistin gene is also expressed in pancreatic islets (12), pre-adipocytes, (14) macrophages, (15) and bone marrow (39). So, resistin is of relevance for inflammation processes as well as for lipid metabolism.

Most investigation refers to mouse models. Here, the existence of trimeric and hexameric resistin in serum was demonstrated (7). Compared to adiponectin biology, it is highly probable that different resistin oligomers have different biologic function (8, 9). In mice, a correlation between adiposity, insulin resistance, and resistin expression was found empirically. In humans, study results are not clear – several studies show an association of resistin serum concentration and adiposity or insulin resistance (17, 25-31). But others failed in confirming these results (14, 16-24). Therefore, there is requirement for valid and reproducible determination of resistin serum concentration.

Relevance of resistin to physiologic processes beyond energy metabolism was investigated by several different approaches. Experiments with endothelial cells gave interesting results. Here, resistin was shown to enhance expression of VCAM-1 and ICAM-1 (33, 34). So, resistin may be able to influence endothelial inflammation (35, 36) and, thereby atherosclerosis. These results were verified by experiments in mice, where endothelin-1 was shown to regulate resistin secretion (37, 38).

In recent research human resistin was shown to increase pre-adipocyte proliferation and lipolysis of mature adipocytes (38). By the way of modulating MAPK-signaling pathways resistin exerts crucial influence on energy metabolism.

3. PRINCIPLE

The enzyme immunoassay for resistin is a so-called sandwich assay. It utilizes a specific high affinity polyclonal rabbit antiserum coated on the wells of a microtiter plate. Resistin in samples binds quantitatively to the immobilized antiserum. In the following step, the biotinylated detecting antiserum binds in turn to resistin. After washing, Streptavidin-Peroxidase Enzyme conjugate will be added, and will bind to the biotin on the bound detecting antibody. In the closing substrate

reaction, a color change will be catalyzed quantitatively depending on the resistin level of the samples.

4. WARNINGS AND PRECAUTIONS

For research use only. Not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. A Material Safety Data sheet is available on request. Temperature will affect the absorbance readings of the assay. However, calculated concentration values for the samples should not be affected. Do not use expired reagents; obviously damaged, microbial contaminated, or spilled material.

Use separate pipette tips for each sample, control, and reagent to avoid cross-contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir that had previously been used for the conjugate solution may turn the solution color. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

Caution: This kit contains material of human and/or animal origin. Therefore, all components and samples should be treated as potentially infectious.

Proper precautions and good laboratory practices must be used in storing, handling, and disposal of kit reagents. Disposal of kit components must be made according to the local regulations.

Human Serum
The following components contain human serum: **Controls CTR1** and **CTR2**
Source human serum for the Controls provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore, all components and specimens should be treated as potentially infectious.

Reagents CAL A-E, DET, EC, SB, DIL, WB
Contain as preservative a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (<0.015%)
H317 May cause an allergic skin reaction.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272 Contaminated work clothing should not be allowed out of the workplace.
P261 Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
P333+P313 If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352 IF ON SKIN: Wash with plenty of soap and water.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Substrate S
The TMB-Substrate S contains 3,3´,5,5´ Tetramethylbenzidine (<0.05%)
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.
P261 Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
P305+P351+ IF IN EYES: Rinse cautiously with water for several minutes.
P338 Remove contact lenses, if present and easy to do. Continue rinsing.

Stop Solution STP
The Stop solution contains 0.2 M acid sulfuric acid (H_2SO_4)
H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+ IF SWALLOWED: rinse mouth.
P331 Do NOT induce vomiting.
P305+P351+ IF IN EYES: Rinse cautiously with water for several minutes.
P338 Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310 IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

4.1 General first aid procedures
Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated clothes and shoes.
Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes, spreading the eyelids to ensure effective rinsing.
Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

5. MATERIALS
5.1 Materials Provided
The reagents listed below are sufficient for 96 wells including the standard curve

MTP	**Microtiter plate**, ready for use, coated with rabbit-anti-resistin-antibody.	**8 x 12 Wells**
CAL A-E	**Calibrators,** lyophilized, (recombinant human Resistin), concentrations are given on vial labels and on quality certificate. **Reconstitute in 750 µL Sample Buffer SB.**	**5 x 750 µL**
CTR 1 & 2	**Control 1 & 2,** lyophilized, (human serum), concentration is given on quality certificate. **Reconstitute in 250 µL Dilution Buffer DIL. Dilute with Sample Buffer SB.**	**1 x 250 µL (Ctrl 1)** **1 x 250 µL (Ctrl 2)**
DET	**Antibody Conjugate, 100-fold concentrated solution,** contains rabbit biotinylated anti-resistin antibody. Before use dilute 1:100 with **Dilution Buffer DIL**:	**1 x 120 µL**
EC	**Enzyme Conjugate,100-fold concentrated solution,** contains HRP (Horseradish peroxidase)-labelled Streptavidin. Before use dilute 1:100 with **Dilution Buffer DIL**:	**1 x 120 µL**
SB	**Sample Buffer,** ready for use.	**1 x 120 mL**
DIL	**Dilution Buffer,** ready for use	**1 x 25 mL**
WB	**Washing Buffer,** 20-fold concentrated solution. Dilute 1:20 in DI water.	**1 x 50 mL**
S	**Substrate,** ready for use, horseradish peroxidase-(HRP) substrate, stabilized tetramethylbenzidine	**1 x 12 mL**
STP	**Stop Solution,** ready for use, 0.2 M sulfuric acid	**1 x 12 mL**
-	Sealing tape for covering of the microtiter plate	**3 X**

5.2 MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes and multichannel pipettes with disposable plastic tips
Graduated cylinder for diluting Wash Buffer **(WB)**
Distilled or deionized water for dilution of the Wash Buffer **(WB)**, 950 mL
Vortex-mixer
Microtiter plate Shaker (350 rpm)
Microtiter plate washer (recommended)
Microplate reader ("ELISA-Reader") with filter for 450 and ≥ 590 nm
Polyethylene PE/Polypropylene PP tubes for dilution of samples
Centrifuge
Timer

6. SAMPLES

6.1 Sample Type

Serum as well plasma samples are suitable. Significant deviation of resistin levels in corresponding serum, heparin plasma, EDTA plasma, and citrate plasma samples were not found. A possible dilution of the sample by the anticoagulant must be taken into account.

By means of the special sample buffer an external sample preparation prior to the assay is not required (see below).

6.2 Sample Collection

The blood sample for serum preparation should be obtained according to standardized venipuncture procedure. The samples should be stored without anticoagulation reagents. Hemolysis must be avoided. The blood must be allowed to clot and after complete clotting, the serum should be separated by centrifugation.

6.3 Required Sample Volume: 15 µL

6.4 Storage of the samples

In firmly closable plastic tube.

Storage at 20 - 25 °C	max. 2 days
Storage at –20°C	min. 2 years

The storage of samples, over a period of 2 years at -20°C, showed no effect on the measured value. Freeze/thaw cycles of samples should be minimized.

6.5 Interference

Triglycerides, bilirubin, and hemolysate in the samples were tested at a concentration of 100 mg/mL, 100 µg/mL, and 1 mg/mL, respectively. Hemolyzed samples appear to show falsely high resistin levels, using such samples should be checked critically (see **Table 1**). The use of hemolyzed, lipemic, or icteric samples should be validated by the user.

6.6 Serum and Plasma Sample Dilution

Dilution: 1:21 with Sample Buffer (**SB**).

Pipette 300 µL Sample Buffer SB in PE-/PP-Tubes (application of multi-stepper is recommended in larger series), add 15 µL serum or plasma sample (dilution 1:21). After mixing, two 100 µL samples can be used to assess the sample in duplicate in the assay.

Dilution may need to be adjusted according to expected resistin levels. Samples may be diluted between 1:5 and 1:400 (based on linearity, see **Table 5**).

The sample buffer is formulated to allow for the correct determination of resistin. **A minimum dilution of 1:5** with sample buffer is recommended for samples!

7. TECHNICAL NOTES

Storage Conditions
Store the kit at 2-8 °C after receipt until its expiry date. The lyophilized reagents should be stored at - 20°C after reconstitution. Avoid repeated thawing and freezing.

Storage Life
The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8 °C in the clip-lock bag, use in the frame provided. The reconstituted components: **Calibrators A – E** and Controls **CTR1** and **CTR2** must be stored at -20 °C (max. 4 weeks). For further use, thaw quickly, but gently(avoid temperature increases above room temperature and avoid excessive vortexing). The 1:20 diluted Wash Buffer **WB** is stable for 4 weeks at 2 – 8 °C.

Preparation of Reagents
Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitates in the buffers must be dissolved before usage by mixing and/or warming. Reagents with different lot numbers cannot be mixed.

Reconstitution
The **Calibrators A-E** are reconstituted with the Sample Buffer **SB.**
The controls **CTR1** and **CTR2** are reconstituted with the dilution buffer **DIL.**
It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam) with a Vortex mixer.
The reconstituted calibrators and controls can be stored up to 4 weeks at –20°C. Repeated freeze/thaw cycles must be avoided.

Dilution
After reconstitution, dilute the Controls **CTR1** and **CTR2** with the Sample Buffer **SB** in the same ratio (1:21) as the samples.

The required volume of washing buffer **WB** is prepared by 1:20 dilution of the provided 20-fold concentrate with deionized water. The diluted wash buffer is stable for 4 weeks at 2-8 °C and should be prepared fresh daily.

Antibody and Enzyme Conjugate
Use the Dilution Buffer DIL for the dilution of the Antibody Conjugate DET and Enzyme Conjugate EC 100-fold concentrates. The diluted solutions have limited stability at 2-8 °C, and should be prepared fresh daily.

Incubation
Incubation at room temperature means incubation at 20-25°C. The substrate **S**, stabilized tetramethylbenzidine, is photosensitive and should be stored and incubated in the dark.

Shaking
The incubation steps are recommended to be performed at 350 rpm and should be performed at mean rotation frequency of a microtiter plate. Differences in plate shaker models may require an adjustment of the shake rate. Insufficient shaking may lead to inadequate mixing of the solutions

and thereby to low optical densities, high variations and/or false values, excessive shaking may result in high optical densities and/or false values.

Washing
Proper washing is of basic importance for a secure, reliable, and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be random variations of measured optical densities, potentially leading to falsely calculated sample values. Effects like high background values or high variations may indicate washing problems. All washing must be performed with the provided wash buffer diluted to usage concentration. Wash volume per wash cycle and well must be at least 300 µl.

When using an **automatic microtiter** plate washer, the respective instructions for use must be carefully followed. Device adjustments, e.g., for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, the fluid remaining in each well should be removed by inverting the plate and repeatedly tapping it dry on lint-free absorbent tissue.

Manual washing is an adequate alternative option. Wash Buffer may be dispensed with a squirt bottle. The fluid may be removed by dynamically swinging out the microtiter plate over a basin. If aspirating devices are used, care must be taken that the inside well surface is not scratched. After every single wash step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on lint-free absorbent tissue.

8. ASSAY PROCEDURE

NOTES: All determinations (Calibrators, Controls, and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended. When performing the assay, the Calibrators, Controls, and the samples should be pipetted as fast as possible (e.g., <15 minutes). To avoid distortions due to differences in incubation times, **Antibody-Conjugate DET** and the **Enzyme Conjugate EC** as well as the following **Substrate Solution S** should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution STP** should be added to the plate in the same order as the **Substrate Solution S**.

1) Add **100 µL Sample Buffer SB** in wells A1/A2 (blank) and

2) Pipette in positions B1/2 **100 µL of the Calibrator A** (0.02 ng/mL),
pipette in positions C1/2 **100 µL of the Calibrator B** (0.1 ng/mL),
pipette in positions D1/2 **100 µL of the Calibrator C** (0.3 ng/mL),
pipette in positions E1/2 **100 µL of the Calibrator D** (0.6 ng/mL),
pipette in positions F1/2 **100 µL of the Calibrator E** (1 ng/mL).

3) Pipette **100 µL** of the controls, previously diluted 1:21 in sample buffer **SB,** (or respective dilution of the sample) into positions G1/2 and H1/2.

4) Pipette **100 µL** each of the **diluted samples** (e.g. dilute 1:21 with Sample Buffer **SB)** into the rest of the wells, according to requirements.

5) Cover the wells with sealing tape and incubate the plate for **2 hours** at **room temperature** (shake at 350 rpm). After incubation aspirate the contents of the wells and wash the wells 5 times with **300 µL 1X Working Wash Buffer WB** / well.

6) Following the last washing step pipette **100 µL** of the 1X Working **Antibody Conjugate DET** in each well. Cover the wells with the sealing tape and incubate **1 hour** at room temperature (shake at 350 rpm).

7) After incubation wash the wells 5 times with **Wash Buffer WB** as described in step 5.

8) Following the last washing step, pipette **100 µL** of the 1X Working **Enzyme Conjugate EC** (diluted with **Dilution Buffer DIL**) into each well. Cover the wells with the sealing tape and incubate for **30 minutes** at room temperature (shake at 350 rpm).

9) After incubation wash the wells 5 times with 1X Working Wash Buffer **WB** as described in the step 5.

10) Pipette **100 µl** of the **TMB-substrate** solution **S** in each well.

11) Incubate the plate for **30 minutes** in the dark at **room temperature**.

12) Stop the reaction by adding **100 µL** of **Stop Solution STP** to all wells.

13) Measure the absorbance within **30 minutes** at **450 nm** (reference filter: ≥ 590nm).

9. ESTABLISHING THE CALIBRATION CURVE

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.25, those of calibrator E should be above 1.0. Samples which yield higher absorbance values than calibrator E should be retested with a higher dilution.
The calibrators provided contain the following concentrations of resistin:

Calibrator	**A**	**B**	**C**	**D**	**E**
ng/mL	0.02	0.10	0.30	0.60	1.00
pg/mL	20	100	300	600	1000

1) Calculate the mean absorbance value for the blank from the duplicated determination (well A1/A2).

2) Subtract the mean absorbance of the blank from the mean absorbances of all other well values.

3) Plot the calibrator concentrations on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis.

4) Recommendation: Calculation of the standard curve should be done by using a computer program because the curve is in general (without respective transformation) not ideally described by linear regression. **A four parametric logistic (4-PL) curve fit, higher-grade polynomial,** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).

5) The **resistin concentration** of the diluted sample or the diluted control sera in ng/mL (or pg/mL according to the chosen unit for the calibrators) is calculated in this way, the resistin concentrations of the **undiluted samples** and of controls are calculated **by multiplication with the respective dilution factor**.

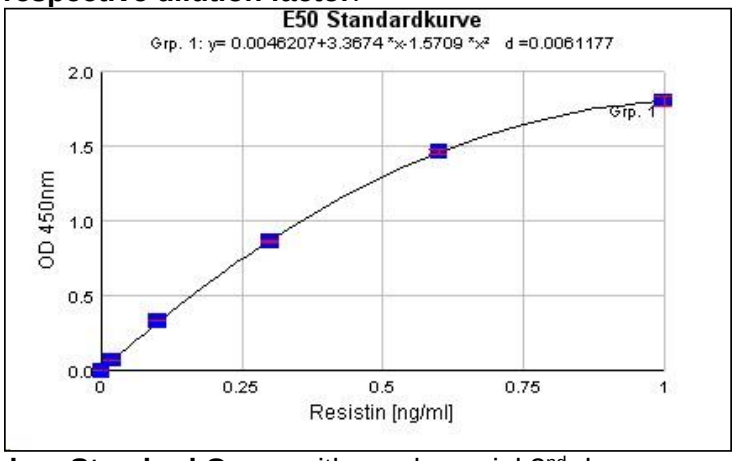

Fig. 1. Exemplary Standard Curve with a polynomial 2nd degree as curve fit.

The exemplary standard curve shown in Fig.1 **cannot** be used for calculation of the test results. Establish a standard curve for each test conducted!

Exemplary calculation of the resistin concentration of a 1:21 diluted sample:

Measured extinction of your sample	0.85
Measured extinction of the blank	0.05

A measurement program will calculate the resistin concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. Just determine the most suitable curve fit (here: polynomial 2nd degree).

In this exemplary case the following equation is solved by the program to calculate the resistin concentration in the sample:

$$y = 0.0046207 + 3.3674\,x - 1.5709x^2$$
$$0.2686 = x$$

If the dilution factor (1:21) is considered, the resistin concentration of the undiluted sample is:
0.2686 x 21 = 5.64 ng/mL = 0.00564 µg/mL

10. PERFORMANCE CHARACTERISTICS

10.1 Calibrators

The calibrators are prepared from recombinant human resistin (19.5 kDa, 2 x 92 amino acids, expressed in E. coli) in concentrations of 20, 100, 300, 600 and 1000 pg/mL (equal to 0.02 ng/mL-1 ng/mL).

10.2 Sensitivity

The **analytical sensitivity** of the assay yields **0.012 ng/mL** (12 pg/mL; as 2x SD of zero calibrator)

10.3 Specificity
Commercially available sera from bovine, cat, chicken, dog, donkey, goat, guinea pig, horse, mouse, pig, rabbit, rat, and sheep were diluted (1:10) and used as samples in this assay system and the signal intensity was measured. No cross reactivity was detected.

10.4 Interference
Interference of physiological appearing substance with the resistin measurement was investigated. Serum samples have been enriched with different concentrations of possibly interfering substances and the amount of resistin was measured and compared with the Resistin concentration in the same sample without any enrichment. In table 1 the relative results are shown. None of the tested substances interfered significantly with resistin measurement.

Table 1: Interference: Three serum samples where enriched with indicated amount of the potentially interfering substance and measured. Shown is % of resistin of the native, non-enriched serum sample

	Triglyceride 100 mg/mL	**Bilirubin 100 µg/mL**	**Hemolysate 1 mg/mL**
Serum 1	101	93	94
Serum 2	115	99	99
Serum 3	104	103	147

Table 2: Effects of coagulation inhibitors were investigating by adding indicated amounts of inhibitors to **SB** enriched with 0.3 ng/mL resistin. Relative amounts of resistin measured in inhibitor containing samples in comparison to 0.3 ng/mL resistin containing Sample Buffer **(SB)** are shown.

		% of Resistin in SB	
		Mean (n=3)	**SD**
3.8 g/L	Citrate	94	7.67
0.0068 mol/L	EDTA	93	4.96
30,000 IU/L	Heparin	96	4.89

10.5 Reproducibility and Precision
The inter- and intra assay coefficients of variability are below 10%. Exemplary determinations are shown in table 3 and table 4.

Table 3: Inter-Assay-Variation

	Number of determinations	**Mean value (ng/mL)**	**Standard deviation (ng/mL)**	**CV (%)**
Sample 1	26	2.91	0.16	5.55
Sample 2	15	4.58	0.24	5.33
Sample 3	17	4.60	0.23	5.04
Sample 4	7	2.50	0.09	3.37
Sample 5	23	4.09	0.27	6.67

Table 4: Intra-Assay-Variation

	Number of determinations	Mean value (ng/mL)	Standard deviation (ng/mL)	CV(%)
Sample 1	16	2.81	0.13	4.49
Sample 2	15	4.79	0.24	4.97

10.6 Recovery and Linearity
The linearity of serum dilutions over a very wide range was excellent (Table 5).

Table 5: Recovery and linearity of the Sample Dilution (characteristic results of two different sera)

Dilution	**Sample 1** (native 5.5 ng/mL)		**Sample 2** (native 2.25 ng/mL)	
	plus 5 ng/mL	Recovery (%)	plus 12.25 ng/mL	Recovery (%)
1:50	9.71	92.5	14.99	103.4
1:100	10.60	101.0	13.64	94.1
1:200	10.44	99.4	14.10	97.2
1:400	10.32	98.3	14.33	98.8

Different human sera were spiked with recombinant human resistin in varying concentrations (e.g. in Table 5). The recovery of resistin yielded on average 98% of the theoretically expected amount.

11. LITERATURE

1. Aruna B, Ghosh S, Singh AK, Mande SC, Srinivas V, Chauhan R, Ehtesham NZ. Human recombinant resistin protein displays a tendency to aggregate by forming intermolecular disulfide linkages. Biochemistry 2003;42(36):10554-9.
2. Steppan CM, Lazar MA. Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab 2002;13(1):18-23.
3. Yang RZ, Huang Q, Xu A, McLenithan JC, Eisen JA, Shuldiner AR, Alkan S, Gong DW, Eison JA. Comparative studies of resistin expression and phylogenomics in human and mouse. Biochem Biophys Res Commun 2003;310(3):927-35.
4. Banerjee RR, Lazar MA. Resistin: molecular history and prognosis. J Mol Med 2003;81(4):218-26.
5. Banerjee RR, Lazar MA. Dimerization of resistin and resistin-like molecules is determined by a single cysteine. J Biol Chem 2001;276(28):25970-3.
6. Raghu P, Ghosh S, Soundarya K, Haseeb A, Aruna B, Ehtesham NZ. Dimerization of human recombinant resistin involves covalent and noncovalent interactions. Biochem Biophys Res Commun 2004;313(3):642-6.
7. Patel SD, Rajala MW, Rossetti L, Scherer PE, Shapiro L. Disulfide-dependent multimeric assembly of resistin family hormones. Science 2004;304(5674):1154-8.
8. Kim KH, Zhao L, Moon Y, Kang C, Sul HS. Dominant inhibitory adipocyte-specific secretory factor (ADSF)/resistin enhances adipogenesis and improves insulin sensitivity. Proc Natl Acad Sci U S A 2004;101(17):6780-5.
9. Chen J, Wang L, Boeg YS, Xia B, Wang J. Differential dimerization and association among resistin family proteins with implications for functional specificity. J Endocrinol 2002;175(2):499-504.
10. 0.Beltowski J. Adiponectin and resistin--new hormones of white adipose tissue. Med Sci Monit 2003;9(2):RA55-61.
11. Morash BA, Ur E, Wiesner G, Roy J, Wilkinson M. Pituitary resistin gene expression: effects of age, gender and obesity. Neuroendocrinology 2004;79(3):149-56.

12. Minn AH, Patterson NB, Pack S, Hoffmann SC, Gavrilova O, Vinson C, Harlan DM, Shalev A. Resistin is expressed in pancreatic i slets. Biochem Biophys Res Commun 2003;310(2):641-5.
13. Fain JN, Cheema PS, Bahouth SW, Lloyd Hiler M. Resistin release by human adipose tissue explants in primary culture. Biochem Biophys Res Commun 2003;300(3):674-8.
14. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res 2002;10(1):1-5.
15. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, Smith SA. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 2003;300(2):472-6.
16. Youn BS, Yu KY, Park HJ, Lee NS, Min SS, Youn MY, Cho YM, Park YJ, Kim SY, Lee HK, Park KS. Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. J Clin Endocrinol Metab 2004;89(1):150-6.
17. Chen XD, Lei T, Xia T, Gan L, Yang ZQ. Increased expression of resistin and tumour necrosis factor-alpha in pig adipose tissue as well as effect of feeding treatment on resistin and cAMP pathway. Diabetes Obes Metab 2004;6(4):271-9.
18. Volarova de Courten B, Degawa-Yamauchi M, Considine RV, Tataranni PA. High serum resistin is associated with an increase in adiposity but not a worsening of insulin resistance in Pima Indians. Diabetes 2004;53(5):1279-84.
19. Shalev A, Patterson NB, Hirshberg B, Rother KI, Harlan DM. Resistin serum levels in type 1 diabetes pre- and post-islet transplantation. Metabolism 2004;53(4):403-4.
20. Fujinami A, Obayashi H, Ohta K, Ichimura T, Nishimura M, Matsui H, Kawahara Y, Yamazaki M, Ogata M, Hasegawa G, Nakamura N, Yoshikawa T, Nakano K, Ohta M. Enzyme-linked immunosorbent assay for circulating human resistin: resistin concentrations in normal subjects and patients with type 2 diabetes. Clin Chim Acta 2004;339(1-2):57-63.
21. Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, Considine RV. Serum resistin (FIZZ3) protein isincreased in obese humans. J Clin Endocrinol Metab 2003;88(11):5452-5.
22. Azuma K, Katsukawa F, Oguchi S, Murata M, Yamazaki H, Shimada A, Saruta T. Correlation between serum resistin level and adiposity in obese individuals. Obes Res 2003;11(8):997-1001.
23. Wang H, Chu WS, Hemphill C, Elbein SC. Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. J Clin Endocrinol Metab 2002;87(6):25204.
24. Brichard SM, Delporte ML, Lambert M. Adipocytokines in anorexia nervosa: a review focusing on leptin and adiponectin. Horm Metab Res 2003;35(6):337-42.
25. Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, Manabe I, Utsunomiya K, Nagai R. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokineendothelial cell interactions. Biochem Biophys Res Commun 2004;314(2):415-9.
26. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, Mickle DA. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation 2003;108(6):736-40.
27. Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR. Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. Biochem Biophys Res Commun 2003;309(2):286-90.
28. Lu SC, Shieh WY, Chen CY, Hsu SC, Chen HL. Lipopolysaccharide increases resistin gene expression in vivo and in vitro. FEBS Lett 2002;530(1-3):158-62.
29. Zhong Q, Lin CY, Clarke KJ, Kemppainen RJ, Schwartz DD, Judd RL. Endothelin-1 inhibits resistin secretion in 3T3-L1 adipocytes. Biochem Biophys Res Commun 2002;296(2):383-7.
30. Barton M, Carmona R, Ortmann J, Krieger JE, Traupe T. Obesity-associated activation of angiotensin and endothelin in the cardiovascular system. Int J Biochem Cell Biol 2003;35(6):826-37.

31. Ort TA, A. A.; MacDougall, J.R.;Nelson, P.J.; Rothenberg, M.E.; Wu, F.; Eisen, A.; Halvorsen, Y.-D.C. Recombiant human FIZZ3/Resistin Stimulates Lipolysis in Cultured Human Adipocytes, Mouse Adipose Explants and in Normal Mice. Endocrinology 2005;doi:10.1210/en.2004-1421.

12. SUMMARY – RESISTIN ELISA

Reagent preparation:	**Reconstitution:**	**Dilution:**
Calibrators A – E	in **750 µl** Sample Buffer **SB**	
Control Serum CTR1	in **250 µl** Dilution Buffer **DIL**	**1:21** with Sample Buffer **SB**
Control Serum CTR2	in **250 µl** Dilution Buffer **DIL**	**1:21** with Sample Buffer **SB**
Antibody Conjugate DET		**1:100** with Dilution Buffer **DIL**
Enzyme Conjugate EC		**1:100** with Dilution Buffer **DIL**
Wash Buffer WB Concentrate		**1:20** with DI H_2O.
Sample dilution: **1:21** Sample Buffer **SB**, mix immediately.		
Before assay procedure bring all reagents to room temperature (20 – 25 °C).		

Assay Procedure for Double Determination

Pipette	**Reagents**	**Position**
100 µl	Sample Buffer **SB** (blank value)	A1/2
100 µl	Calibrator **A** (0.02 ng/ml)	B1/2
100 µl	Calibrator **B** (0.1 ng/ml)	C1/2
100 µl	Calibrator **C** (0.3 ng/ml)	D1/2
100 µl	Calibrator **D** (0.6 ng/ml)	E1/2
100 µl	Calibrator **E** (1.0 ng/ml)	F1/2
100 µl	Control Serum **CTR1** (1:21 diluted)	G1/2
100 µl	Control Serum **CTR2** (1:21 diluted)	H1/2
100 µl	Sample dilution (1:21 diluted)	in the rest of the wells according to requirements
Cover the wells with the sealing tape.		
Incubation: 2 h at 20 -25 °C, 350 rpm		
5x 300 µl	Aspirate the contents of wells and wash 5x with **300 µl** 1X Wash Buffer **WB**	each well
100 µl	**1X Working** Antibody Conjugate **DET**	each well
Cover the wells with the sealing tape.		
Incubation: 1 hr at 20 -25 °C, 350 rpm		
5x 300 µl	Aspirate the contents of wells and wash 5x with **300 µl** 1X Wash Buffer **WB**	each well
100 µl	**1X Working** Enzyme Conjugate **EC**	each well
Cover the wells with the sealing tape.		
Incubation: 30 minutes at 20 -25 °C, 350 rpm		
5x 300 µl	Aspirate the contents of wells and wash 5x with **300 µl** 1X Wash Buffer **WB**	each well
100 µl	Substrate Solution **S**	each well
Incubation: 30 minutes at 20 -25 °C in the dark		
100 µl	Stop Solution **STP**	each well
Measure the absorbance within **30 min** at **450 nm** with **≥590 nm** as reference wavelength		