Resistin Elisa

KAPME50

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Resistin-ELISA

Enzyme Immunoassay for the Quantitive Determination of human Resistin

KAPME50

IN VITRO DIAGNOSTIC USE

CE

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DIASOURCE RESISTIN ELISA

- is suited for Resistin determination in **Serum** and **Plasma** samples
- is extremely **sensitive** (**12 pg/ml** ≅ **1.2 pg per well**) and, thus allows measurements in cell culture media too and in specimens others than serum e.g. in Cerebrospinal fluid, Amnion fluid, Saliva, Urine, Breast milk
- is **fast**: incubation time a total of 4 hours
- Single Calibrators with **20, 100, 300, 600,1000 pg/ml** human Resistin are provided in the Kit
- 2 Control Sera for quality control
- is calibrated with **recombinant Resistin**
- Microtiter plates are separately breakapart, tests can be adapted to individual requirements

INTENDED USE

Measurement of human Resistin in human Serum and Plasma Sample

CLINICAL IMPLICATION

- Resistin is relevant e.g. in research of:
- Adiposity
- Insulin Resistance, Diabetes
- Arteriosclerosis
- Inflammation

INTRODUCTION

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

In humans, resistin and RELMβ (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 secreted as a dimer, build by 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). Further on, the resistin structure seems to be dependent on its concentration, as circular dichroism analysis shows a concentration dependent shift of α-helical to β-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. But in vitro, resistin expression of non-adipocytes in fatty tissue was shown (13). Human resistin gene is also expressed in pancreatic islets (12), pre-adipocytes (14 marcophages (15) and bone marrow (39). So, resistin is of relevance for inflammation processes as well as for lipid metabolism.

Most investigation refers to the mouse model. Here, the existence of trimeric and hexameric resistin in serum was demonstrated (7). In comparison 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, respective 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 in other physiologic processes than 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). By this way, resistin is potentially able to influence endothelial inflammation (35, 36) and, thereby atherosclerosis. These results were confirmed by experiments in mice, where endothelin-1 was shown to regulate resistin secretion (37, 38).

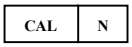
In recent research human resistin was shown to increase pre-adipocyte proliferation and lipolysis of mature adipocytes (38). By the way of modulating MAPK-signalling pathways resistin exerts crucial influence on energy metabolism.

Present research demonstrates, that Resistin exerts influence on a broad variety of physiological processes, however a clear and defined biological role of resistin remains still unexisting.

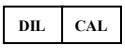
This ELISA-kit enables the user to determine the exact concentration of Resistin in human serum/plasma as well as other body fluids and thereby assists investigation of Resistin biology.

REAGENTS PROVIDED

1)  **Microtiter plate**, ready for use: **Microtiter plate** with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with anti-human Resistin antibody.

2) 

Calibrators 1-5, lyophilized: contain recombinant Resistin. Calibrator values are between **0.02 - 1 ng/ml** (20, 100, 300, 600 und 1000 pg/ml) Resistin and have to be reconstituted with **750 µl (each) Calibrator Diluent**. Attention: Please use only Calibrator Diluent for this dilution, because only this assures, that the Calibrators and the respective samples subsequently will incubate under identical conditions in the same special buffer!

3) 

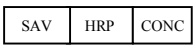
Calibrator Diluent, 120 ml, ready for use, please use for the reconstitution of the Calibrators 1 – 5 and for the sample and Control 1 dilution.

4) 

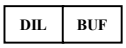
Controls 1 and 2, lyophilised: Contain human Serum and have to be reconstituted with **250 µl Dilution buffer**. The Resistin target value concentration and the respective range is given on the vial label. The **dilution** of the **Control 1 and 2** in **Calibrator Diluent** should be according done to the dilution of the respected samples.

5) 

Biotin Conjugate, 120 µl, 100-fold concentrated solution, contains biotinylated anti-Resistin antibody, please dilute before use 1:100 in Dilution buffer: e.g., add 100 µl Biotin Conjugate to 10 ml Dilution Buffer, mix and use 100 µl/well of this dilution in the assay.

6) 

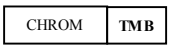
HRP Conjugate, 120 µl, 100-fold concentrated solution, contains HRP (Horseradish peroxidase)-labelled Streptavidin, please dilute before use **1:100** in **Dilution Buffer :** e.g. add 100 µl HRP conjugate to 10 ml Dilution buffer, mix and use 100 µl/well of this dilution in the assay.

7) 

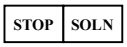
Dilution buffer, 25 ml, ready for use, please use this for the **reconstitution** of **the Controls** and for the **dilution** of **Biotin Conjugate** and **HRP Conjugate**.

8) 

Washing Buffer, 50 ml, 20-fold concentrated: Washing Buffer has to be diluted 1:20 with distilled or demineralised water before use. (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill with A.dest. to 1000 ml). Attention: After dilution, the Washing Buffer is only limited stable, please dilute only according to requirements.

9) 

Chromogenic Substrate, 12 ml, ready for use, stabilised H_2O_2-Tetramethylbencidine.

10) 

Stopping Solution, 12 ml, ready for use, 0,2 M sulphuric acid, *Caution!*

11) **Sealing tape** for covering of the microtiter plate**,** 2 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes (100 and 200µl) Micropipettes and multichannel pipettes with disposable plastic tips
Distilled or Deionized water for dilution of the Washing Buffer (WP), 950 ml
Vortex-mixer
Device to aspirate the calibrators and the samples from the wells (recommended because of the potential danger of infection by human samples)
Timer (120 min. range)
Reservoirs (disposable)
Plate washer and plate shaker (350 cpm)
Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620nm (or ≥590 nm)

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic use only. For professional use only.
Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

Before use, all kit components should be brought **to room temperature at 20 - 25°C**. Precipitates in buffers should be dissolved before use by thorough mixing and warming. **Temperature will affect the absorbance** readings of the assay. However, values for the patient samples will not be affected.

Do not mix reagents of different lots. Do not use expired reagents.

The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.

Caution: This kit contains material of human and/or animal origin. Source human serum for the Control Serum provided in this kit was tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibodies. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

2-Methyl-4-Isothiazolin-3-one
Following components contain < 0.01% **2-Methyl-4-isothiazolin-3-one** solution as preservative **Calibrator 1-5, Biotin Conjugate, Calibrator Diluent, Enzyme conjugate, Dilution buffer**

< 0.01% 2-Methyl-4-isothiazolin-3-one Solution

R36/38	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin wash immediately with plenty of water

5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one
Following components contain < 0.01%(w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-isothiazol-3-one as preservative: **Calibrator 1-5, Biotin Conjugate, Calibrator Diluent, Washing Buffer, Enzyme Conjugate, Dilution Buffer**

R36/38	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice S28.1 S28.1 After contact with skin, wash immediately with plenty of water

Stop solution contains 0.2 M Sulfuric Acid (H_2SO_4)

R36/38	Irritating to eyes and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves.

Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. 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 for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. 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.

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine. Store and incubate in the dark.

R20/21/R22	Harmful by inhalation, in contact with skin and if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves

General first aid procedures:
Skin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.
Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.
Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.
Do not eat, drink or smoke in these areas.
Never pipette the materials with the mouth.
Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

METHOD
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. The Resistin in the samples binds quantitatively to the immobilized antiserum. In the following step, the biotinylated antiserum binds in turn to Resistin. After washing, Streptavidin-Peroxidase-Enzyme conjugate will be added, which will bind highly specific to the biotin of the antiserum and will catalyse in the closing substrate reaction the turn of the colour, quantitatively depending on the Resistin level of the samples.

SPECIMEN
Serum as well plasma samples are suitable (significant deviation of Resistin levels in corresponding serum-, Heparin-, EDTA-, Citrate-plasma-Samples were not found). Haemolytic samples appear to show falsely high Resistin levels, using such samples should be checked out critically. Common cell culture medium, saliva, breast milk and urine were found to be suitable specimens too.
By means of the special sample buffer an external sample preparation prior to the assay is not required (see below).

The blood sample for serum preparation should be gained according to calibratorized venipuncture procedure. The samples should be stored without anticoagulation reagents. Haemolytic reactions have to be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation.

Storage of the samples

Storage at RT max. 2 days

Storage at –20°C max. 2 years

in tightly closable plastic tubes.

More than 3 freeze/thaw cycles are not possible.

Sample Preparation

Samples have to be diluted in Calibrator Diluent. The excellent linearity of this test system allows sample dilution of 1:5 to 1:400.

In most determinations (serum or plasma samples, and no extreme values expected) a dilution **from 1:10 to 1:50 with Calibrator Diluent** should be suitable. According to expected Resistin levels the dilution with Calibrator Diluent can be higher or lower. Because the Calibrator Diluent has a special formulation for the correct determination of Resistin, the dilution should be **at least 1:5**!
Resistin concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants.

For clinical purposes we recommend a standard dilution of 1:21.

Suggestion for dilution protocol:
Pipette 300 µl Calibrator Diluent in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 15 µl Serum- or Plasma (dilution1:21). After mixing use 2 x 100 µl of this dilution in the assay.

TECHNICAL RECOMMENDATIONS

The assay has to be conducted strictly according the test protocol herein.

Reagents with different lot numbers cannot be mixed. The microtiterplate and reagents are stable until the indicated expiry if stored unopened and protected from sunlight at 2 – 8°C.

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming.

Incubation at room temperature means: 20-25°C
The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must become adjusted. 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.

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 uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

All washing must be performed with the provided washing buffer diluted to usage concentration. Washing volume per washing cycle and well must be 300 µl at least.

The danger of handling with potentially infectious material must be taken into account.

When using an automatic microtiter plate washer, the respective instructions fur 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, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamically swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

Calibrators and Control

For the reconstitution of the lyophilised **Calibrators 1 – 5**, **Calibrator Diluent** has to be used.

The lyophilised **Controls** must be reconstituted with the **Dilution Buffer**. The dilution of the **Control in Calibrator Diluent** should be done according the dilution of the respected samples.

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 calibrator and controls can be stored for 4 weeks at –20°C. Repeated freeze/thaw cycles have to be avoided.

Biotin and HRP Conjugate

Use the Dilution Buffer for the dilution of the Biotin Conjugate and HRP Conjugate 100fold concentrates. The diluted solutions are only limited stable at 2-8°C and should be prepared daily fresh.

Washing Buffer

The required volume of washing buffer is prepared by 1:20 dilution of the provided 20fold concentrate with deionised water. The diluted Washing Buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage!

Microtiterplate

Store the once unused microtiter strips and wells together with the desiccant in the tightly closed clip lock bag at 2-8°C use in the frame provided. The labelled expiry is not influenced in case of proper storage.

Chromogenic Substrate

The Chromogenic Substrate, stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

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 pipette as fast as possible (e.g., <15 minutes). To avoid differences in incubation times, **Biotin Conjugate** and the **HRP Conjugate** as well as the following **Chromogenic Substrate** should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution** should be added to the plate in the same order as the Substrate Solution.

1) Add **100 µl Calibrator Diluent** in wells A1/A2 (blank) and

2) Pipette in positions B1/2 **100 µl of the Calibrator 1** (0.02 ng/ml)
Pipette in positions C1/2 **100 µl of the Calibrator 2** (0.1 ng/ml),
Pipette in positions D1/2 **100 µl of the Calibrator 3** (0.3 ng/ml),
Pipette in positions E1/2 **100 µl of the Calibrator 4** (0.6 ng/ml),
Pipette in positions F1/2 **100 µl of the Calibrator 5** (1 ng/ml).

3) To control the correct accomplishment 100 µl of the 1:21 (or in respective dilution rate of the sample) **in Calibrator Diluent** diluted **Controls 1 and 2** can be pipetted in positions G1/2.
Pipette **100 µl** each of the **diluted sample** (e.g. dilute 1:21 with Calibrator Diluent**)** in the rest of the wells, according to requirements.

4) 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 Washing buffer** / well.

5) Following the last washing step pipette **100 µl** of the 1:100 with **Dilution buffer** diluted **Biotin Conjugate** in each well and incubate **1 hour** at **room temperature** (shake at ≥350 rpm).

6) After incubation wash the wells 5 times with **Washing Buffer** as described in step 4)

7) Following the last washing step, pipette **100 µl** of the 1:100 with Dilution Buffer diluted **HRP Conjugate** in each well and incubate the plate for **30 minutes** at **room temperature** (shake at ≥350 rpm).

8) After incubation wash the wells 5 times with Washing Buffer as described in the step 4.

9) Pipette **100 µl** of the **TMB-Chromogenic substrate** in each well.

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

11) Stop the reaction by adding **100 µl** of **Stopping Solution** to all wells.

12) Measure the absorbance within **30 minutes** at **450 nm** (reference filter: 620 nm).

ESTABLISHING THE CALIBRATION CURVE

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.3 OD, these of calibrator 5 should exceed 0.8 OD.

Samples, which yield higher absorbance values than Calibrator 5 are beyond the calibration curve, for reliable determinations these samples should be tested again with a higher dilution.

The calibrators provided contain the following concentrations of Resistin:

Calibrator	**1**	**2**	**3**	**4**	**5**
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 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 calibration 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 higher-grade polynomial,** or **four parametric logistic (4-PL) curve fit** 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 in ng/ml (or µg/ml according the chosen unit for the calibrators) is calculated in this way, the Resistin concentration of the **undiluted sample** and of control is calculated **by multiplication with the respective dilution factor**.

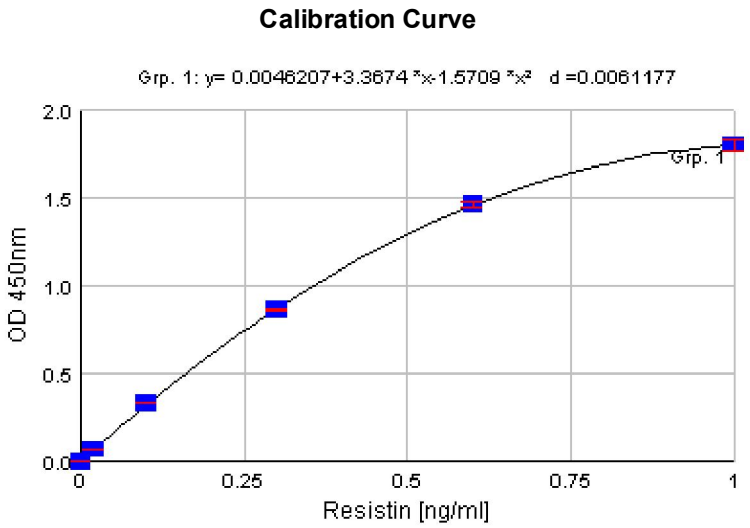


Fig. 1. Exemplary Calibration Curve with a polynomial 2nd degree as curve fit.

The exemplary shown calibration curve in Fig.1 **cannot** be used for calculation of your test results. You have to establish a calibration curve for each test you conduct!
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

Your measurement program will calculate the Resistin concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. You only have to 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 taken into account the Resistin concentration of the undiluted sample is
$0.2686 \times 21 = 5{,}64$ ng/mL = 0.00564 µg/mL

PERFORMANCE CHARACTERISTICS

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 (pico Gramm / ml, equal to 0.02 ng/ml-1 ng/ml).

Sensitivity

The **analytical sensitivity** of the assay yields **0.012 ng/ml** (12 pg/ml; as 2x SD of zero calibrator in 15fold determination).

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.

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**	**Haemolysate 1000 µg/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 PP 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 (PP) are shown.

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

Reproducibility and Precision

The inter- and intra assay coefficients of variability are below than 6.8% and 5%, respectively.
Exemplary determinations are shown in table 3 and table 4.

Table 3: Intra-Assay-Variation

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	VC (%)
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: Inter-Assay-Variation (results of 11 determinations, each)

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

Recovery and Linearity

The DIAsource Resistin ELISA is over a very wide range dilution authentic, the linearity of serum dilutions is over a very wide range excellent (s.Tab.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 6). The recovery of Resistin yielded on average 98 % of the theoretically expected amount.

Table 6: Samples were enriched with 0.3 ng/ml Resistin and measured in comparison to non enriched sample. Relative recovery of added Resistin is shown.

Matrix	**Dilution**	**% Recovery**
Cerebrospinal fluid	1:2	129
Cerebrospinal fluid	1:10	93
Cerebrospinal fluid	1:40	103
Amnion fluid	1:10	85
Amnion fluid	1:40	91
Saliva	1:10	99
Saliva	1:21	86
Urine	1:10	79
Urine	1:21	85
Breast milk	1:2	97
Breast milk	1:10	58
Breast milk	1:21	63
Cell culture supernatant	1:2	100

EVALUATION OF RESULTS

Table 7: The expected values for Resistin were determined with the DIAsource ELISA in healthy probands and analysed by Prof. Dr. J. Kratzsch, Institute for Laboratory Medicine, University of Leipzig.

Female				**Resistin (ng/ml):**		
Age (Years):	**n:**	**AV Age:**	**AV BMI:**	**AV ± SD:**	**25.- 75. Percentile:**	**Min. – Max.:**
18 - 30	96	23.0	23.1	**7.2 ±** 2.6	5.4 – 8.8	3.1 – 14.7
31 - 40	63	36.5	24.3	**8.1 ±** 2.3	6.4 – 9.6	3.6 – 13.1
41 - 50	67	44.9	24.8	**7.3 ±** 2.5	5.7 – 8.1	4.0 – 16.1
51 - 60	29	54.7	25.0	**7.2 ±** 2.6	5.4 – 8.5	4.0 – 15.5
61 - 65	9	62.7	25.2	**6.6 ±** 1.1	6.0 – 6.7	5.4 – 9.3
Male				**Resistin (ng/ml):**		
Age (Years):	**n:**	**AV Age:**	**AV BMI:**	**AV ± SD:**	**25.- 75. Percentile:**	**Min. – Max.:**
18 - 30	107	23.9	24.1	**6.4 ±** 1,8	5.0 – 7.6	2.5 – 13.1
31 - 40	59	35.9	25.0	**6.7 ±** 3,2	4.8 – 7.4	3.8 – 26.9
41 - 50	66	45.0	25.2	**6.5 ±** 2,8	4.5 – 7.4	2.4 – 16.7
51 - 60	36	54.8	26.4	**6.1 ±** 2,1	4.7 – 7.2	3.2 – 13.3
61 - 68	20	63.2	25.6	**7.2 ±** 1,8	6.0 – 8.2	4.5 – 11.2

n=Number of Probands, **AV=**Average Value, **BMI=**Body Mass Index (kg/m²), **SD=**Standard Deviation

Table 8: Summary of the expected values

Sex	**Number**	**Mean [ng/ml]**	**Standard deviation**	**2.5. Percentile**	**9.5. Percentile**
Male	288	6.48	2.44	3.32	11.68
Female	264	7.41	2.47	3.68	13.60
Total	552	6.93	2.49	3.58	13.12

LITERATUR / LITERATURE

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Summary of the Assay

Reagent preparation:	Reconstitution:	Dilution:
Calibrators 1 – 5	in **750 µl** Calibrator Diluent	
Control 1	in **250 µl** Dilution Buffer	**1:21** with Calibrator Diluent
Control 2	in **250 µl** Dilution Buffer	**1:21** with Calibrator Diluent
Biotin Conjugate		**1:100** with Dilution Buffer
HRP Conjugate		**1:100** with Dilution Buffer
Washing Buffer		**1:20** with **Aqua. dest.** (e.g., add the complete contents of the flask **(50 ml)** into a graduated flask and fill with A.dest. to 1000 ml).
Sample dilution: **1:21** (e.g. 15 µl Serum with 300 µl Calibrator Diluent).		

Assay Procedure for Double Determination

Pipette	Reagents	Position
100 µl	Calibrator Dilulent (blank value)	A1/2
100 µl	Calibrator **1** (0.02 ng/ml)	B1/2
100 µl	Calibrator **2** (0.1 ng/ml)	C1/2
100 µl	Calibrator **3** (0.3 ng/ml)	D1/2
100 µl	Calibrator **4** (0.6 ng/ml)	E1/2
100 µl	Calibrator **5** (1.0 ng/ml)	F1/2
100 µl	Control 1 (diluted)	G1/2
100 µl	Control 2 (diluted)	H1/2
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
Incubation: 2 h at **RT, ≥ 350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 3x with **300 µl** Wash Buffer	each well
100 µl	**1:100** diluted Biotin Conjugate	each well
Incubation: 1 h at **RT, ≥350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 3x with **300 µl** Wash Buffer	each well
100 µl	**1:100** diluted HRP Conjugate	each well
Incubation: 30 min at **RT, ≥350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 3x with **300 µl** Wash Buffer	each well
100 µl	Chromogenic Substrate	each well
Incubation: 30 min in the dark at **RT**		
100 µl	Stop Solution	each well
Measure the absorbance within **30 min** at **450 nm** with **620 nm** as reference wavelength.		

Revision date : 2013-05-06

	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
IVD	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoadsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor
TRAY	Incubation trays
PMSF	PMSF solution
	Protect from light
STRIP	Dot Strip
SUB	Substrate
EXTR SOLN CONC	Extraction Buffer Concentrate
CART	Cartridge
SAV HRP	Streptavidin HRP
WASH SOLN	Wash buffer