

Resistin - ELISA

Enzyme Immunoassay for Quantitative Determination
of

human Resistin

Produkt-Code: E50
(96 Determinations)

For EU:



DE/CA40/00809/14

For EU: For in-vitro diagnostic use
Worldwide except EU: For Research Use Only!



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CLINICAL IMPLICATIONS

Resistin is relevant e.g. in research of:

Adiposity

Insulin Resistance, Diabetes

Arteriosclerosis

Inflammation

TECHNICAL FEATURES

The **Resistin ELISA E50**:

- is suited for determination in serum and plasma samples

contains optimised special sample buffer: reliable quantification of resistin - irrespective of absolute concentration

- is extremely sensitive (**6 pg/ml \cong 0.6 pg per well**) and, thus allows measurements in cell culture media too and in specimens others than serum

- is fast: incubation time a total of 4 hours

- is calibrated with recombinant Resistin:

single standards with 20, 100, 300, 600, 1000 pg/ml are provided in the kit

Control serum KS of human serum

INTRODUCTION

Resistin, a cysteine-rich protein of 11,3 kDa (1), was firstly found in mice (2) and constitutes together with RELM α , RELM β and RELM γ the protein family of resistin-like molecules (RELM).

In humans, Resistin and RELM β (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 propeptide 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) macrophages (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 exert 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.

INTENDED USE

The Mediagnost ELISA-Kit E50 aims by means of precise determination of resistin level in human serum or other sample matrices at clarifying the potential function of resistin, in particular in research fields like:

Adiposity
Insulin Resistance, Diabetes
Arteriosclerosis
Inflammation

METHODOLOGY

Assay Characteristics

The enzyme immunoassay for Resistin E50 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, a 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.

The standards are prepared from recombinant human resistin dimers (19,5 kD, 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).

Different human sera were spiked with recombinant human Resistin in varying concentrations (e.g. in Table 1). The recovery of resistin yielded on average 95 % of the theoretically expected amount.

Table 1: 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

The **analytical sensitivity** of the assay yields **0,006 ng/ml** (6 pg/ml; as 2x SD of zero standard in 15fold determination).

The **Inter-** and **Intra-Assay** Variations-coefficients were found **less than 6,8% and 5% respectively**. Exemplary determinations are shown in table 2 and table 3.

Table 2: Inter-Assay-Variation

(results of 11 determinations, each)

	Mean value (ng/ml)	Standard deviation (ng/ml)	VK (%)
Sample 1	2.70	0.16	5.94
Sample 2	4.20	0.28	6.77
Sample 3	5.80	0.28	4.79

Table 3: Intra-Assay-Variation

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

Samples: Applicability, Preparation and Storage

Serum as well plasma samples are suitable (significant deviation of Resistin levels in corresponding Serum-, Heparin plasma-, EDTA-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 was found to be

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

Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination store the undiluted samples frozen at -20°C or below in tightly

closable plastic tubes. Avoid on principle repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote) although levels were found to be unaffected by few cycles.

The high sensitivity of the assay allows measurement of Resistin in small sample volumes, which is limited by pipetting accuracy rather than the amount of Resistin.

In most determinations (serum or plasma samples, and no extreme values expected) a dilution **from 1:10 to 1:50 with Sample Buffer PP** should be suitable. According to expected Resistin levels the dilution with PP can be higher or lower. Because the sample buffer PP is special composed for the correct determination of Resistin, the dilution should be **at least 1:5!**

In general, a dilution factor of **1:21** for serum or plasma samples is appropriate. Resistin concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants.

Suggestion for dilution protocol:

Pipette 300 μl Sample Buffer PP in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 15 μl Serum- or Plasma (dilution 1:21). After mixing use 2 x 100 μl of this dilution in the assay.

MATERIALS

Materials 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 and packed in a laminate bag.
- 2) **Standards A-E**, lyophilized: contain recombinant Resistin. Standard 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) Sample Buffer PP**. Attention: Please use only Sample buffer PP for this dilution, because only this assures, that the Standards and the respective samples subsequently will incubate under identical conditions in the same special buffer!
- 3) **Sample Buffer PP**, 120 ml, ready for use, please use for the reconstitution of the Standards A – E and for the sample and Control Serum KS dilution.
- 4) **Control Serum KS**, lyophilised: Contains human Serum and has to be reconstituted with **100 µl Dilution buffer VP**. The Resistin target value concentration and the respective range is given on the vial label. The **dilution** of the **Control Serum KS** in **Sample Buffer PP** should be according to the dilution of the respected samples.
- 5) **Antibody Conjugate AK**, 120 µl, 100-fold concentrated solution, contains biotinylated anti-Resistin antibody, please dilute before use 1:100 in Dilution buffer VP:
e.g., add 100 µl Antibody Conjugate AK to 10 ml Dilution Buffer VP, mix and use 100 µl/well of this dilution in the assay.

- 6) **Enzyme Conjugate EK**, 120 µl, 100-fold concentrated solution, contains HRP (Horseradish peroxidase)-labelled Streptavidin, please dilute before use **1:100** in **Dilution Buffer VP**: e.g. add 100 µl Enzyme conjugate EK to 10 ml Dilution buffer VP, mix and use 100 µl/well of this dilution in the assay.
- 7) **Dilution buffer VP**, 25 ml, ready for use, please use this for the **reconstitution** of **Control Serum KS** and for the **dilution** of **Antibody Conjugate AK** and **Enzyme Conjugate EK**.
- 8) **Washing Buffer WP**, 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) **Substrate S**, 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H₂O₂-Tetramethylbencidine.
- 10) **Stopping Solution SL**, 12 ml, ready for use, 0,2 M sulphuric acid, *Caution!*
- 11) **Sealing tape** for covering of the microtiter plate, 2 x, adhesive.

TECHNICAL RECOMMENDATIONS

In conducting the assay, follow strictly the test protocol.

Reagents with different lot numbers should not be mixed.

The Microtiter Plate and all reagents are stable until the expiry date if stored in the dark at 2-8°C (s. label).

For the **reconstitution** of the lyophilised components the kit **Dilution Buffer VP (for the Control serum KS)** and **Sample Buffer PP (for the Standards A - E)** respectively should be used. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

The shelf life of the components after opening is not affected, if used appropriately.

Store the unused seal stripes of the microtiter plate together with the desiccant at 2-8°C.

After reconstitution the components (**Standards A – E** and **Control Serum KS**) should be stored at 2-8°C for up to 1 week. If longer storage time is needed, store the components frozen at -20°C or below. Freezing extends the expiry at least 2 months. Avoid repeated freeze-thaw cycles. In case you plan to perform multiple independent determinations over a longer period with one kit, you should aliquot the components prior to freezing into suitable smaller volumes. This is strongly recommended. The 1:20 diluted **Washing Buffer WP** is only limited stable. Please dilute only according to requirements. This applies to the 1:100 **Antibody Conjugate AK** and **Enzyme Conjugate EK** dilutions too.

Before use, all kit components should be brought **to room temperature**. Room temperature incubation means: incubation at 20 - 25°C. Precipitates in buffers should be dissolved before use through mixing and warming.

The **Substrate Solution S**, stabilised H₂O₂-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

When performing the assay, the **Standards (A-E)**, **Control Serum (KS)** 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 (AK)** and the **Enzyme Conjugate (EK)** as well as the succeeding **Substrate Solution** should be added to the plate in the same order and in the same time interval as the **samples**. **Stop Solution (SL)** should be added to the plate in the same order as the Substrate Solution.

Materials not Provided

Distilled or demineralised water for dilution of the Washing buffer WP

Micropipettes and multichannel pipettes with disposable plastic tips

Vortex-mixer

Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human Samples)

Plate washer and plate shaker (recommended)

Microplate reader ("ELISA-Reader") with filter for 450/620nm(or ≥ 590 nm).

Foil welding device for laminate bags (recommended)

PRECAUTIONS

The **mediagnost Resistin ELISA, E50** is for in-vitro use only! This product has to be used as described in the enclosed instructions. The Mediagnost GmbH is not liable for any loss or harm caused by non-observance of the instructions, as far as no law withstands.

Caution: This kit contains material of human and/or animal origin. All components have to be treated as potentially infectious.

Reasonable precautions have to be taken and rules of good laboratory practice are to apply regarding storage, use and waste disposal of all kit components. Waste disposal has to be done in accordance with local regulations.

Human Serum:

contained in following components: **KS**

Human material, used for preparation of this products was tested by regulatory accredited methods for antibodies against human immunodeficient virus (HIV I and II), against hepatitis B virus surface antigen and against hepatitis C virus and shown as negative for all tested antibodies. No test method exclude the presence of infectious pathogens totally, therefore all reagent should be treated according the guidelines of biological safety level 2.

Reagents contain as preservatives

ProClin 950

Following components contain ProCline 950 : **AK, EK, VP, PP**

< 0,1% 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

Kathon CG

Following components contain Kathon CG **AK, EK, VP, WP, PP**

< 0,1% (w/w) 5-chloro-2-methyl 2H isothiazol-3-one und 2-methyl-2H-isothiazol-3-one

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 (H₂SO₄)

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.

TMB-Substrate (S) contains 3,3',5,5' Tetramethylbenzidine. Store and Incubate in tge 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

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

The handling of potentially infectious material must comply with the following guidelines:

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.

PROCEDURE

All determinations (Standards, Control Serum and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

- 1) Add **100 µl Sample Buffer PP** in wells A1/A2 (blank) and
- 2) Pipette in positions B1/2 **100 µl of the Standard A** (0.02 ng/ml)
Pipette in positions C1/2 **100 µl of the Standard B** (0.1 ng/ml),
Pipette in positions D1/2 **100 µl of the Standard C** (0.3 ng/ml),

Pipette in positions E1/2 **100 µl of the Standard D** (0.6 ng/ml),

Pipette in positions F1/2 **100 µl of the Standard E** (1 ng/ml).

To control the correct accomplishment 100 µl of the 1:21 (or in respective dilution rate of the sample) in **Sample Buffer PP** diluted **Control Serum KS** can be pipetted in positions G1/2.

Pipette **100 µl** each of the **diluted sample** (e.g. dilute 1:21 with Sample Buffer **PP**) in the rest of the wells, according to requirements.

- 3) Cover the wells with sealing tape and incubate the plate for **2 hours at room temperature** (if possible, shake at ≥ 350 rpm) After incubation aspirate the contents of the wells and wash the wells 3 times with **250 µl Washing buffer WP** / well.
- 4) Following the last washing step pipette **100 µl** of the 1:100 with **Dilution buffer VP** diluted **Antibody Conjugate AK** in each well and incubate **1 hour at room temperature** (if possible shake at ≥ 350 rpm).
- 5) After incubation wash the wells 3 times with **Washing Buffer WP** as described in step 4)
- 6) Following the last washing step, pipette **100 µl** of the 1:100 with Dilution Buffer VP diluted **Enzyme Conjugate EK** in each well and incubate the plate for **30 minutes at room temperature** (if possible shake at ≥ 350 rpm).
- 7) After incubation wash the wells 3 times with Washing Buffer **WP** as described in the step 4
- 8) Pipette **100 µl** of the **TMB-substrate** solution **S** in each well.
- 9) Incubate the plate for **30 minutes** in the dark at **room temperature**.
- 10) Stop the reaction by adding **100 µl** of **Stopping Solution SL** to all wells.

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

EVALUATION OF RESULTS

Expected values

Table 4: The expected values for Resistin were determined with the Mediagnost ELISA E50 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

Establishing the Standard Curve

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

Samples, which yield higher absorbance values than Standard E are beyond the standard curve, for reliable determinations these samples should be tested anew with a higher dilution.

The standards provided contain the following concentrations of Resistin:

Standard	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 (MA) value for the blank from the duplicated determination (well A1/A2).
- 2) Subtract the mean absorbance (MA) of the blank from the mean absorbances of all other values
- 3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
- 4) Recommendation: Calculation of standard curve should be done by using a computer programme, because the curve is in general (without respective transformation) not ideally described by linear regression. A higher-grade polynomial or four parameter logistic (4PL) lin-log curve fit are suitable for the evaluation.
- 5) The Resistin concentration in ng/ml (or in pg/ml according the chosen unit for the standards) of the samples can be calculated by multiplication with the respective dilution factor.

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Summary of the Assay

Reagent preparation:	Reconstitution:	Dilution:
Standards A – E	in 750 µl Sample Buffer PP	
Control Serum KS	in 100 µl Dilution Buffer VP	1:21 with Sample Buffer PP
Antibody Conjugate AK		1:100 with Dilution Buffer VP
Enzyme Conjugate EK		1:100 with Dilution Buffer VP
Washing Buffer WP		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 Sample Buffer **PP**) .

Assay Procedure for Double Determination

Pipette	Reagents	Position
100 µl	Sample Puffer PP (blank value)	A1/2
100 µl	Standard A (0.02 ng/ml)	B1/2
100 µl	Standard B (0.1 ng/ml)	C1/2
100 µl	Standard C (0.3 ng/ml)	D1/2
100 µl	Standard D (0.6 ng/ml)	E1/2
100 µl	Standard E (1.0 ng/ml)	F1/2
100 µl	Control Serum KS	G1/2
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
Incubation: 2 h at RT, ≥ 350 rpm		
3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer WP	each well
100 µl	1:100 diluted Antibody Conjugate AK	each well
Incubation: 1 h at RT, ≥350 rpm		
3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer WP	each well
100 µl	1:100 diluted Enzyme Conjugate EK	each well
Incubation: 30 min at RT, ≥350 rpm		
3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer WP	each well
100 µl	Substrate Solution S	each well
Incubation: 30 min in the dark at RT		
100 µl	Stop Solution SL	each well
Measure the absorbance within 30 min at 450 nm with 620 nm as reference wavelength.		