



Resistin Elisa

KAPME50



Resistin-ELISA

Enzyme Immunoassay for the Quantitative Determination of human Resistin
KAPME50
IN VITRO DIAGNOSTIC USE

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DIASOURCE RESISTIN ELISA

- is suited for Resistin determination in **Serum** and **Plasma** samples
- is extremely **sensitive (12 pg/ml $\pm$ 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 ng/ml** human Resistin are provided in the Kit
- Control Serum is of human serum
- 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 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 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)

CAL	N
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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)

DIL	CAL
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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)

CONTROL	1
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Control 1, lyophilised: Contains human Serum and has to be reconstituted with **100 µl Dilution buffer**. The Resistin target value concentration and the respective range is given on the vial label. The **dilution** of the **Control 1** in **Calibrator Diluent** should be according done to the dilution of the respected samples.
- 5)

BIOT	CONJ	CONC
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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)

SAV	HRP	CONC
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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)

DIL	BUF
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Dilution buffer, 25 ml, ready for use, please use this for the **reconstitution** of **Control 1** and for the **dilution** of **Biotin Conjugate** and **HRP Conjugate**.
- 8)

WASH	SOLN	CONC
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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)

CHROM	TMB
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Chromogenic Substrate, 12 ml, ready for use, stabilised H₂O₂-Tetramethylbenzidine.
- 10)

STOP	SOLN
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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)
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 (recommended)
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**

< 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

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.

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 (dilution 1:21). After mixing use 2 x 100 µl of this dilution in the assay.

TECHNICAL RECOMMENDATIONS

The assay has to be conducted strictly according to 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

Calibrators and Control

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

The lyophilised **Control** must be reconstituted with the **Dilution Buffer**. The dilution of the **Control in Calibrator Diluent** should be done according to 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 2 months 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.

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 H₂O₂-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

ASSAY PROCEDURE

NOTES: All determinations (Calibrators, Control 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, Control 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).

To control the correct accomplishment 100 µl of the 1:21 (or in respective dilution rate of the sample) in **Calibrator Diluent** diluted **Control 1** 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.

- 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** / well.
- 4) 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** (if possible shake at ≥ 350 rpm).
- 5) After incubation wash the wells 3 times with **Washing Buffer** as described in step 3)
- 6) 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** (if possible shake at ≥ 350 rpm).
- 7) After incubation wash the wells 3 times with Washing Buffer as described in the step 3.
- 8) Pipette **100 µl** of the **TMB-Chromogenic substrate** 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** to all wells.
- 11) 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**.

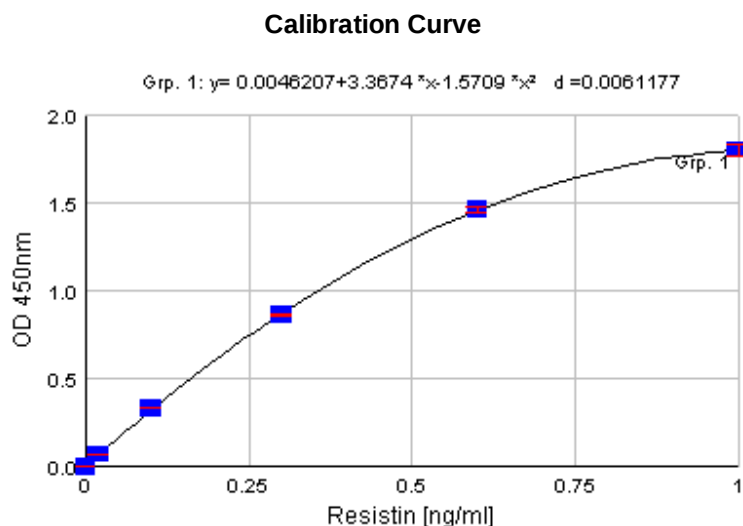


Fig. 1. Exemplary Calibration Curve with a polynomial 2nd degree as curve fit.

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:

$$y = 0.0046207 + 3.3674x - 1.5709x^2$$

$$0.2686 = x$$

if the dilution factor (1:21) is taken into account the Resistin concentration of the undiluted sample is

$$0.2686 \times 21 = 5.64 \text{ ng/mL} = 0.00564 \text{ } \mu\text{g/mL}$$

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	Bilirubin	Haemolysate
	100 mg/ml	100 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$
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 (µg/ml)	Standard deviation (µg/ml)	CV (%)
Sample 1	16	5.87	0.138	2.35
Sample 2	16	12.19	0.377	3.10
Sample 3	6	14.36	0.668	4.66

Table 4: Inter-Assay-Variation (results of 11 determinations, each)

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

Recovery and Linearity

The DIALsource 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 $\pm$ SD:	25.- 75. Percentile:	Min. – Max.:
18 - 30	96	23.0	23.1	7.2 $\pm$ 2.6	5.4 – 8.8	3.1 – 14.7
31 - 40	63	36.5	24.3	8.1 $\pm$ 2.3	6.4 – 9.6	3.6 – 13.1
41 - 50	67	44.9	24.8	7.3 $\pm$ 2.5	5.7 – 8.1	4.0 – 16.1
51 - 60	29	54.7	25.0	7.2 $\pm$ 2.6	5.4 – 8.5	4.0 – 15.5
61 - 65	9	62.7	25.2	6.6 $\pm$ 1.1	6.0 – 6.7	5.4 – 9.3
Male				Resistin (ng/ml):		
Age (Years):	n:	AV Age:	AV BMI:	AV $\pm$ SD:	25.- 75. Percentile:	Min. – Max.:
18 - 30	107	23.9	24.1	6.4 $\pm$ 1.8	5.0 – 7.6	2.5 – 13.1
31 - 40	59	35.9	25.0	6.7 $\pm$ 3.2	4.8 – 7.4	3.8 – 26.9
41 - 50	66	45.0	25.2	6.5 $\pm$ 2.8	4.5 – 7.4	2.4 – 16.7
51 - 60	36	54.8	26.4	6.1 $\pm$ 2.1	4.7 – 7.2	3.2 – 13.3
61 - 68	20	63.2	25.6	7.2 $\pm$ 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 100 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 Diluent (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	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	each well
100 µl	1:100 diluted Biotin Conjugate	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	each well
100 µl	1:100 diluted HRP Conjugate	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	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 : 2010-02-25

	<u>Used symbols</u>	<u>Symboles utilisés</u>
	Consult instructions for use	Consulter les instructions d'utilisation
	Storage temperature	Température de conservation
	Use by	Utiliser jusque
	Batch code	Numéro de lot
	Catalogue number	Référence de catalogue
	Control	Contrôle
	In vitro diagnostic medical device	Dispositif médical de diagnostic in vitro
	Manufacturer	Fabricant
	Contains sufficient for <n> tests	Contenu suffisant pour <n> tests
	Wash solution concentrated	Solution de lavage concentrée
	Zero calibrator	Calibrateur zéro
	Calibrator #	Calibrateur #
	Control #	Contrôle #
	Tracer	Traceur
	Tracer	Traceur
	Tracer concentrated	Traceur concentré
	Tracer concentrated	Traceur concentré
	Tubes	Tubes
	Incubation buffer	Tampon d'incubation
	Acetonitrile	Acétonitrile
	Serum	Sérum
	Specimen diluent	Diluant du spécimen
	Dilution buffer	Tampon de dilution
	Antiserum	Antisérum
	Immunoabsorbent	Immunoabsorbant
	Calibrator diluent	Diluant de calibrateur
	Reconstitution solution	Solution de reconstitution
	Polyethylene glycol	Glycol Polyéthylène
	Extraction solution	Solution d'extraction
	Elution solution	Solution d'élution
	Bond Elut Silica cartridges	Cartouches Bond Elut Silica
	Pre-treatment solution	Solution de pré-traitement
	Neutralization solution	Solution de neutralisation
	Tracer buffer	Tampon traceur
	Microtiterplate	Microplaque de titration
	HRP Conjugate	HRP Conjugué
	HRP Conjugate	HRP Conjugué
	HRP Conjugate concentrate	HRP Conjugué concentré
	HRP Conjugate concentrate	HRP Conjugué concentré
	Conjugate buffer	Tampon conjugué
	Chromogenic TMB concentrate	Chromogène TMB concentré
	Chromogenic TMB solution	Solution chromogène TMB
	Substrate buffer	Tampon substrat
	Stop solution	Solution d'arrêt
	Incubation serum	Sérum d'incubation
	Buffer	Tampon
	AP Conjugate	AP Conjugué
	Substrate PNPP	Tampon PNPP
	Biotin conjugate concentrate	Biotine conjugué concentré
	Avidine HRP concentrate	Avidine HRP concentré
	Assay buffer	Tampon de test
	Biotin conjugate	Biotine conjugué
	Specific Antibody	Anticorps spécifique
	Streptavidin HRP concentrate	Concentré streptavidine HRP
	Non-specific binding	Liant non spécifique
	2nd Antibody	Second anticorps
	Acidification Buffer	Tampon d'acidification

	<u>Gebruikte symbolen</u>	<u>Gebrauchte Symbolen</u>
	Raadpleeg de gebruiksaanwijzing	Gebrauchsanweisung beachten
	Bewaartemperatuur	Lagern bei
	Houdbaar tot	Verwendbar bis
	Lotnummer	Chargenbezeichnung
	Catalogusnummer	Bestellnummer
	Controle	Kontrolle
	Medisch hulpmiddel voor in-vitro diagnostiek	In Vitro Diagnostikum
	Fabrikant	Hersteller
	Inhoud voldoende voor <n> testen	Ausreichend für <n> Ansätze
	Wasoplossing, geconcentreerd	Waschlösung-Konzentrat
	Nulkalibrator	Null kalibrator
	Kalibrator #	Kalibrator #
	Controle #	Kontrolle #
	Tracer	Tracer
	Tracer	Tracer
	Tracer geconcentreerd	Tracer Konzentrat
	Tracer geconcentreerd	Tracer Konzentrat
	Buisjes	Röhrchen
	Incubatiebuffer	Inkubationspuffer
	Acetonitrile	Azetonitril
	Serum	Humanserum
	Specimen diluent	Probenverdünner
	Verdunningsbuffer	Verdünnungspuffer
	Antiserum	Antiserum
	Immunoabsorbent	Immunadsorbens
	Kalibratorverdunner	Kalibratorverdünnung
	Reconstitutieoplossing	Rekonstitutionslösung
	Polyethyleen glycol	Polyethylenglykol
	Extractieoplossing	Extraktionslösung
	Elutieoplossing	Eluierungslösung
	Bond Elut Silica kolom	Bond Elut Silikakartuschen
	Pre-behandelingsoplossing	Vorbehandlungslösung
	Neutralisatieoplossing	Neutralisierungslösung
	Tracerbuffer	Tracer-Puffer
	Microtiterplaat	Mikrotiterplatte
	HRP Conjugaat	HRP Konjugat
	HRP Conjugaat	HRP Konjugat
	HRP Conjugaat geconcentreerd	HRP Konjugat Konzentrat
	HRP Conjugaat geconcentreerd	HRP Konjugat Konzentrat
	Conjugaat buffer	Konjugatpuffer
	Chromogene TMB geconcentreerd	Chromogenes TMB Konzentrat
	Chromogene Oplossing TMB	Farblösung TMB
	Substraatbuffer	Substratpuffer
	Stopoplossing	Stopplösung
	Incubatieserum	Inkubationsserum
	Buffer	Puffer
	AP Conjugaat	AP Konjugat
	Substraat PNPP	Substrat PNPP
	Geconcentreerd Biotine conjugaat	Biotin-Konjugat-Konzentrat
	Geconcentreerd Avidine-HRP conjugaat	Avidin-HRP-Konzentrat
	Assay buffer	Assaypuffer
	Biotine conjugaat	Biotin-Konjugat
	Specifiek antilichaam	Spezifischer Antikörper
	Streptavidine-HRP concentraat	HRP Streptavidinkonzentrat
	Aspecifieke binding	Unspezifische Bindung
	2de antilichaam	Sekundärer Antikörper
	Verzuringbuffer	Ansäuerungspuffer

	<u>Simboli utilizzati</u>	<u>Símbolos utilizados</u>
	Consultare le istruzioni per l'uso	Consultar las instrucciones de uso
	Limitazioni di temperatura	Limitación de temperatura
	Utilizzare entro	Fecha de caducidad
	Numero di lotto	Código de lote
	Numero di catalogo	Número de catálogo
	Controllo	Control
	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico in vitro
	Fabbricante	Fabricante
	Contenuto sufficiente per <n> saggi	Contenido suficiente para <n> ensayos
	Tampone di lavaggio concentrato	Solución de lavado concentrada
	Calibratore zero	Calibrador cero
	Standard #	Calibrador #
	Controllo #	Control #
	Marcato	Trazador
	Marcato	Trazador
	Marcato concentrato	Trazador concentrada
	Marcato concentrato	Trazador concentrada
	Provette	Tubos
	Tampone incubazione	Tampón de incubación
	Acetonitrile	Acetonitrilo
	Siero	Suero
	Diluente campione	Diluyente de Muestra
	Tampone diluizione	Tampón de dilución
	Antisiero	Antisuero
	Immunoassorbente	Inmunoadsorbente
	Diluente calibratore	Diluyente de calibrador
	Soluzione di ricostituzione	Solución de Reconstitución
	Polietilenglicole	Glicol Polietileno
	Soluzione di estrazione	Solución de extracción
	Soluzione di eluizione	Solución de elución
	Cartucce di silice bond elut	Cartuchos Bond Elut Silica
	Soluzione di pretrattamento	Solución de Pre-tratamiento
	Soluzione di neutralizzazione	Solución de Neutralización
	Tracer Buffer	Tampón de trazador
	Piastra di microtitolazione	Placa de microvaloración
	HRP Coniugato	HRP Conjugado
	HRP Coniugato	HRP Conjugado
	HRP Coniugato concentrato	HRP Conjugado concentrada
	HRP Coniugato concentrato	HRP Conjugado concentrada
	Buffer coniugato	Tampón de Conjugado
	Cromogena TMB concentrato	Cromógena TMB concentrada
	Soluzione cromogena TMB	Solución Cromógena TMB
	Tampone substrato	Tampón de sustrato
	Soluzione di arresto	Solución de Parada
	Incubazione con siero	Suero de Incubación
	Buffer	Tampón
	AP Coniugato	AP Conjugado
	Substrato PNPP	Sustrato PNPP
	Concentrato coniugato con biotina	Concentrado de conjugado de biotina
	Concentrato avidina HRP	Concentrado avidina-HRP
	Soluzione tampone per test	Tampón de ensayo
	Coniugato con biotina	Conjugado de biotina
	Anticorpo Specifico	Anticuerpo específico
	Streptavidina-HRP concentrata	Estreptavidina-HRP Concentrado
	Legame non-specifico	Unión no específica
	2° Anticorpo	Segundo anticuerpo
	Tampone Acidificante	Tampón de Acidificación

	<u>Símbolos utilizados</u>	<u>Använda symboler</u>
	Consulte instruções de utilização	Läs instruktionerna före användning
	Temperatura de conservação	Förvaringstemperatur
	Utilizar antes de	Används av
	Código de lote	Lotnummer
	Número de catálogo	Katalognummer
	Controlo	Kontroll
	Dispositivo médico de diagnóstico in vitro	In vitro diagnostiskt kit
	Fabricante	Tillverkare
	Conteúdo suficiente para <n> testes	Innehållet räcker till <n> prover
	Solução de lavagem concentrada	Tvättlösning, koncentrerad
	Calibrador zero	Nollkalibrerare
	Calibrador #	Kalibrator #
	Controlo #	Kontroll #
	Marcador	Radioisotop, antigen
	Marcador	Radioisotop, antikropp
	Marcador concentrada	Radioisotop, antigen koncentrerad
	Marcador concentrada	Radioisotop, antikropp koncentrerad
	Tubos	Rör
	Tampão de incubação	Inkuberingsbuffert
	Acetonitrilo	Acetonitril
	Soro	Serum
	Diluidor de espécimes	Spädningsbuffert för prover
	Tampão de diluição	Spädningsbuffert
	Anti-soro	Antiserum
	Imunoadsorvente	Immunoadsorberare
	Diluyente do calibrador	Kalibratordiluent
	Solução de Reconstituição	Rekonstitutionslösning
	Polietileno-glicol	Polyetylen glykol
	Solução de Extração	Extraktionslösning
	Solução de Eluição	Elueringslösning
	Cartuchos de sílica Bond Elut	Silikonpatroner för elueringsbindning
	Solução de pré-tratamento	Förbehandlingslösning
	Solução de neutralização	Neutraliseringslösning
	Tampão Marcador	Tracerbuffert
	Placa de micro titulação	Microtiterplatta
	HRP Conjugação	HRP-konjugat
	HRP Conjugação	HRP-konjugat
	HRP Conjugação concentrada	HRP-konjugat-koncentrat
	HRP Conjugação concentrada	HRP-konjugat-koncentrat
	Conjuge o tampão	Konjugatbuffert
	Cromogénica TMB concentrada	Kromogeniskt TMB-koncentrat
	Solução Cromogénica TMB	Kromogenisk TMB-lösning
	Tampão de substrato	Substratbuffert
	Solução de Paragem	Stoplösning
	Soro de incubação	Inkubationsserum
	Tampão	Buffert
	AP Conjugação	AP-konjugat
	Substrato PNPP	Substrat-PNPP
	Concentrado conjugado de biotina	Biotinkonjugat koncentrat
	Concentrado HRP de avidina	Avidin HRP-koncentrat
	Tampão de ensaio	Provbuffert
	Conjugado de biotina	Biotinkonjugat
	Anticorpo específico	-
	Estreptavidina HRP concentrado	-
	Ligações não específicas	-
	Anticorpo secundário	-
	Tampão de acidificação	-

	<u>Επεξήγηση συμβόλων</u>	<u>Anvendte symboler</u>
	Συμβουλευτείτε τις οδηγίες χρήσης	Læs brugsvejledningen
	Θερμοκρασία αποθήκευσης	Opbevaringstemperatur
	Ημερομηνία λήξης	Anvend inden
	Αριθμός παρτίδας	Batchkode
	Αριθμός καταλόγου	Katalognummer
	Πρότυπο ελέγχου	Kontrol
	In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν	Medicinsk udstyr til in vitro-diagnosticering
	Κατασκευαστής	Fabrikant
	Περιεχόμενο επαρκές για «n» εξετάσεις	Indeholder nok til <n> test
	Συμπυκνωμένο διάλυμα έκπλυσης	Koncentreret vaskeopløsning
	Μηδενικός βαθμονομητής	Nul-kalibrator
	Βαθμονομητής #	Kalibrator nr.
	Ορός ελέγχου #	Kontrol nr.
	Ιχνηθέτης	Markør
	Ιχνηθέτης	Markør
	Χρωμογόνος Ιχνηθέτης	Koncentreret markør
	Χρωμογόνος Ιχνηθέτης	Koncentreret markør
	Σοληνάρια	Tuber
	Ρυθμιστικό διάλυμα επώασης	Inkubationsbuffer
	Ακετονιτρίλιο	Acetonitril
	Ορός	Serum
	Διάλυμα αραίωσης δειγμάτων	Prøvediluent
	Ρυθμιστικό διάλυμα αραίωσης	Fortyndingsbuffer
	Αντιορός	Antiserum
	Ανοσοπροσροφητικό	Immonoadsorbent
	Αραιωτικό βαθμονομητών	Kalibratordiluent
	Διάλυμα ανασύστασης	Rekonstitueringsopløsning
	Πολυαιθυλενογλυκόλη	Polyetylenglykol
	Διάλυμα εκχύλισης	Ekstraktionsopløsning
	Διάλυμα έκλουσης	Elueringsopløsning
	Φύσιγγες πυριτίου Bond Elut	Patroner med bindingselueringssilica
	Διάλυμα προεπεξεργασίας	Forbehandlingsopløsning
	Διάλυμα εξουδετέρωσης	Neutraliseringsopløsning
	Ρυθμιστικό διάλυμα	Markørbuffer
	Πλάκα μικροτιτλοδότησης	Mikrotiterplade
	HRP Σύζευγμα	HRP-konjugat
	HRP Σύζευγμα	HRP-konjugat
	Χρωμογόνος HRP Σύζευγμα	HRP-konjugat-koncentreret
	Χρωμογόνος HRP Σύζευγμα	HRP-konjugat-koncentreret
	Ρυθμιστικό διάλυμα συζεύγματος	Konjugatbuffer
	Χρωμογόνος TMB	Kromogen TMB-koncentreret
	Διάλυμα χρωμογόνου TMB	Kromogen TMB-opløsning
	Ρυθμιστικό διάλυμα υποστρώματος	Substratbuffer
	Ανασχετικό αντιδραστήριο	Stopopløsning
	Ορός επώασης	Inkubationsserum
	Ρυθμιστικό διάλυμα	Buffer
	AP Σύζευγμα	AP-konjugat
	PNPP υποστρώματος	Substrat PNPP
	Συμπυκνωμένο αντιδραστήριο συζευγμένο με βιοτίνη	Biotin konjugat koncentrat
	Συμπυκνωμένο διάλυμα αβιδίνης-HRP	Avidin HRP koncentrat
	Ρυθμιστικό διάλυμα προσδιορισμού	Prøvebuffer
	αντιδραστήριο συζευγμένο με βιοτίνη	Biotin konjugat
	Ειδικό Αντίσωμα	-
	Συμπυκνωμένη στρεπταβιδίνη συνεξευγμένη με HRP	-
	μη-ειδική δέσμευση	-
	2ο Αντίσωμα	-
	Ρυθμιστικό Διάλυμα όξινο	-

	<u>Stosowane symbole</u>	<u>Használt szimbólumok</u>
	Przed zastosowaniem zapoznać się z instrukcją	Olvassa el a használati útmutatót
	Temperatura przechowywania	Tárolási hőmérséklet
	Zużyć przed	Lejáratí idő
	Kod serii	Gyártási kód
	Numer katalogowy	Katalógus szám
	Kontrola	Kontrol
	Urządzenie medyczne do diagnostyki in vitro	In vitro diagnosztikai eszköz
	Producent	Gyártó
	Zawartość wystarczająca do <n> testów	Tartalma <n> teszt elvégzésére elegendő
	Roztwór płuczący stężony	Mosó folyadék koncentrátum
	Kalibrator zerowy	Zero kalibrátor
	Kalibrator nr	Kalibrátor #
	Kontrola nr	Kontrol #
	Znacznik izotopowy	Nyomjelző izotóp
	Znacznik izotopowy	Nyomjelző izotóp
	Znacznik izotopowy stężony	Nyomjelző izotóp koncentrátum
	Znacznik izotopowy stężony	Nyomjelző izotóp koncentrátum
	Probówki	Csővek
	Wymagana inkubacja buforu	Inkubáló puffer
	Acetonitryl	Acetonitril
	Surowica	Szérum
	Rozcieńczalnik próbki	Mintahígító
	Bufor do rozcieńczania	Hígító puffer
	Antysurowica	Antiszérum
	Immunoabsorbent	Immunadszorbens
	Rozcieńczalnik kalibratora	Kalibrátor hígító
	Roztwór do rozcieńczania	Mintaelőkészítő oldat
	Glikol poli(oksy)etylenowy	Polietilén glikol
	Roztwór ekstrakcyjny	Extrakciós oldat
	Roztwór elucyjny	Eluáló oldat
	Kolumny krzemionkowe Bond Elut	Bond Elut Silica szilikagél patronok
	Roztwór do przygotowania wstępnego	Előkezelő oldat
	Roztwór neutralizujący	Semlegesítő oldat
	Bufor znacznika	Nyomjelző izotóp hígító puffer
	mikro płytki	Mikrotiter lemez
	Koniugat peroksydazy chrzanowej	HRP konjugátum
	Koniugat peroksydazy chrzanowej	HRP konjugátum
	Koncentrat koniugatu peroksydazy chrzanowej	HRP konjugátum koncentrátum
	Koncentrat koniugatu peroksydazy chrzanowej	HRP konjugátum koncentrátum
	Bufor do koniugacji	Konjugátum puffer
	Koncentrat chromogenu TMB (czterometylobenzodyny)	Kromogén TMB koncentrátum
	Roztwór chromogenu TMB (czterometylobenzodyny)	Kromogén TMB oldat
	Bufor substratu	Szubsztrát puffer
	Roztwór zatrzymujący reakcję	Stop oldat
	Wymagana inkubacja surowicy	Inkubációs szérum
	Bufor	Puffer
	Koniugat AP (fosfatazy alkalicznej)	AP konjugátum
	p-nitrofenylofosforan substratowy	Szubsztrát PNPP
	Koncentrat koniugatu biotyny	Biotin konjugátum koncentrátum
	Koncentrat peroksydazy chrzanowej z awidyną	Avidin HRP koncentrátum
	Bufor do oznaczania	Vizsgálati puffer
	Koniugatu biotyny	Biotin konjugátum
	Przeciwciało swoiste	Specifikus ellenanyag
	Koncentrat streptawidyny HRP	Sztreptawidin HRP koncentrátum
	Wiązanie nieswoiste	Nem-specifikus kötődés
	Drugie przeciwciało	Másodlagos ellenanyag
	Bufor zakwaszający	Savas puffer

	<u>Използвани символи</u>
	Вижте инструкцията за работа
	Температура на съхранение
	Използвайте с
LOT	Партиден код
REF	Каталожен номер
CONTROL	Контрол
IVD	Ин витро диагностично медицинско изделие
	Производител
	Съдържание достатъчно за <n> теста
WASH SOLN CONC	Концентриран измиващ разтвор
CAL 0	Нулев калибратор
CAL N	Калибратор #
CONTROL N	Контрол #
Ag 125I	Трейсър
Ab 125I	Трейсър
Ag 125I CONC	Концентриран маркер
Ab 125I CONC	Концентриран маркер
	Епруветки
INC BUF	Инкубационен буфер
ACETONITRILE	Ацетонитрил
SERUM	Серум
DIL SPE	Разредител за пробите
DIL BUF	Буфер за разреждане
ANTISERUM	Антисерум
IMMUNOABSORBENT	Имуноабсорбент
DIL CAL	Разредител за калибратора
REC SOLN	Пресъздаващ разтвор
PEG	Полиетилен гликол
EXTR SOLN	Екстрактов разтвор
ELU SOLN	Разтвор за елюиране
GEL	Силикагелни пълнители
PRE SOLN	Пред-лечебен разтвор
NEUTR SOLN	Неутрализиращ разтвор
TRACEUR BUF	Маркерен буфер
	Микротитърна пластина
Ab HRP	HRP конюгат / Конюгат на хрянова пероксидаза
Ag HRP	HRP конюгат / Конюгат на хрянова пероксидаза
Ab HRP CONC	HRP конюгиран концентрат
Ag HRP CONC	HRP конюгиран концентрат
CONJ BUF	Буфер за конюгата
CHROM TMB CONC	Хромогенен ТМВ концентрат
CHROM TMB	Хромогенен ТМВ разтвор
SUB BUF	Субстратен буфер
STOP SOLN	Стоп разтвор
INC SER	Инкубационен серум
BUF	Буфер
Ab AP	AP конюгат / конюгат на алкална фосфатаза
SUB PNPP	Субстрат PNPP / пара нитрофенил фосфат
BIOT CONJ CONC	Биотин конюгиран концентрат
AVID HRP CONC	Авидин HRP концентрат
ASS BUF	Буфер за пробите
Ab BIOT	Биотин конюгат
Ab	специфично антитяло
SAV HRP CONC	стрептавидин HRP концентрат
NSB	не специфично свързване
2nd Ab	второ антитяло
ACID BUF	киселинизиращ буфер