Version 18-11/24

User's Manual

Human Resistin ELISA Kit

REF **DEIA-NB24-23**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Human Resistin ELISA Kit is intended to be used for quantitative measurement of human Resistin in human serum or plasma for research purposes.

Due to high sensitivity (12 pg/mL), this assay is also suitable for measurements in cell culture supernatants and in non-serum / plasma samples (e.g. in breast milk, urine, saliva).

General Description

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

In humans, resistin and RELMβ but no other proteins of the RELM family were found. The human form of resistin shows a homology of 53% to the murine protein. 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. Beside this intermolecular disulfide bridge, 5 additional intramolecular ones exist.

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. 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.

Resistin expression was demonstrated in white adipose tissue, pituitary and pancreatic islets 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. Human resistin gene is also expressed in pancreatic islets, pre-adipocytes, marcophages and bone marrow. 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. In comparison to adiponectin biology, it is highly probable that different resistin oligomers have different biologic function.

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. But others failed in confirming these results. 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. By this way, resistin is potentially able to influence endothelial inflammation and, thereby atherosclerosis. These results were confirmed by experiments in mice, where endothelin-1 was shown to regulate resistin secretion.

In recent research human resistin was shown to increase pre-adipocyte proliferation and lipolysis of mature adipocytes. 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. With optimized special sample buffer: reliable quantification of resistin - independent of the absolute content.

Principles of Testing

The Human Resistin ELISA Kit 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.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with rabbit-anti-resistin-antibody. Wells are separately breakable. (8x12) wells
2. **Calibrators (CAL A-E):** lyophilized, (recombinant human Resistin), concentrations are given on vial labels and on quality certificate. Reconstitute in 750 µL Sample Buffer SB. 5 x 750 µL
3. **Control 1 (CTR1):** lyophilized, (human serum), concentration is given on quality certificate. Reconstitute in 250 µL Dilution Buffer DIL. Dilute with Sample Buffer SB. 1 x 250 µL
4. **Control 2 (CTR2):** lyophilized, (human serum), concentration is given on quality certificate. Reconstitute in 250 µL Dilution Buffer DIL. Dilute with Sample Buffer SB. 1 x 250 µL
5. **Antibody Conjugate (DET):** 100-fold concentrated, contains rabbit biotinylated anti-resistin antibody. Before use, dilute 1:100 with Dilution Buffer DIL. 1 x 120 µL
6. **Enzyme Conjugate (EC):** 100-fold concentrated, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin. Before use, dilute 1:100 with Dilution Buffer DIL. 1 x 120 µL
7. **Sample Buffer (SB):** ready for use. Please shake before use! 1 x 120 mL
8. **Dilution Buffer (DIL):** ready for use. Please shake before use! 1 x 25 mL
9. **Washing Buffer (WB):** 20-fold concentrated solution. Dilute 1:20 with Aqua dest. 1 x 50 mL
10. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine. 1 x 12 mL
11. **Stopping Solution (STP):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL
12. **Sealing Tape:** for covering the microtiter plate. 3

Materials Required But Not Supplied

1. Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer WP (A. dest.), 950 mL.
2. Precision pipettes and multichannel pipettes with disposable plastic tips
3. Polyethylene PE/Polypropylene PP tubes for dilution of samples

4. Vortex-mixer
5. Microtiter plate shaker (350 rpm)
6. Microtiter plate washer (recommended)
7. Microplate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

Storage

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

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

Specimen Collection And Preparation

1. Sample type: Serum and plasma

Serum as well plasma samples are suitable (significant deviation of Resistin levels in corresponding serum-, Heparin-, EDTA-, Citrate-plasma-Samples were not found. A possible dilution of the sample by the anticoagulant must be taken into account). Haemolytic samples appear to show falsely high Resistin levels, using such samples should be checked out critically By means of the special sample buffer an external sample preparation prior to the assay is not required.

2. Specimen collection

Use standard venipuncture for the blood sampling. Haemolytic reactions are to be avoided.

3. Required sample volume: 15 µL

4. Sample stability

In firmly closable plastic tubes.

• Storage at 20 -25°C: max. 2 days

• Storage at –20°C min. 2 years

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

5. Interference

Triglyceride, bilirubin and hemolysate in the sample do not interfere to a concentration of 100 mg/mL and 100 µg/mL or 1 mg/mL. However, the use of hemolytic, lipemic or icteric samples should be validated by the user.

6. Sample dilution

• Dilution: 1:21 with Sample Buffer SB

• Pipette 300 µL Sample Buffer SB 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

• According to expected Resistin levels the dilution with SB can be higher or lower. The excellent linearity of this test system allows sample dilution of 1:5 to 1:400.

• Because the Sample Buffer SB is special composed for the correct determination of Resistin, the dilution should be at least 1:5!

Reagent Preparation

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. Reagents with different lot numbers cannot be mixed.

1. The Calibrators A – E is reconstituted with 750 µL Sample Buffer SB. After resuspension, the calibrators are diluted according to a gradient - A (0.02 ng/mL), B (0.1 ng/mL), C (0.3 ng/mL), D (0.6 ng/mL), E (1.0 ng/mL), which are prepared for immediate use.
2. The Controls CTR1 and CTR2 are reconstituted with 250 µL Dilution Buffer DIL. After reconstitution, dilute the Controls CTR1 and CTR2 with the Sample Buffer SB in the same ratio (1:21) as the sample.

Note: 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.

3. The required volume of Washing Buffer WP is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest.
4. Use the Dilution Buffer DIL for the dilution of the Antibody Conjugate DET and Enzyme Conjugate EC 100-fold concentrates. The diluted solutions are only limited stable at 2-8°C and should be prepared daily fresh.

Assay Procedure

Note

1. Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate S, stabilised Tetramethylbencidine, is photosensitive–store and incubation in the dark.
2. When performing the assay, Blank, Calibrators A-E, Controls CTR1 and CTR2 and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate DET and the Enzyme Conjugate EC as well as the succeeding Substrate S should be added to the plate in the same order and in the same time interval as the samples. Stop Solution STP should be added to the plate in the same order as Substrate S. All determinations (Blank, Calibrators A-E, Controls CTR1 and CTR2 and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.
3. Shaking: The incubation steps should be performed at mean rotation frequency of a microtiter plate shaker. We recommend 350 rpm. Depending on the design of the shaker, the shaking frequency should be 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.
4. 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 WB diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.

Automatic washing: 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: Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamical 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.

Assay Step

1. Pipette in positions A1/2 100 µl Sample Buffer SB (blank value).
2. Pipette in positions B1/B2 100 µl Calibrator A (0.02 ng/mL).

   Pipette in positions C1/C2 100 µl Calibrator B (0.1 ng/mL).

   Pipette in positions D1/D2 100 µl Calibrator C (0.3 ng/mL).

   Pipette in positions E1/E2 100 µl Calibrator D (0.6 ng/mL).

   Pipette in positions F1/F2 100 µl Calibrator E (1.0 ng/mL).
3. Pipette in positions G1/G2 100 µl of the Control CTR1 (1:21 diluted) and pipette in positions H1/H2 100 µl of the Control CTR2 (1:21 diluted).
4. Add 100 µl Sample (1:21 diluted) in the rest of the wells according to requirements.
5. Cover the wells with sealing tape and incubate the plate for 2 hours at 20-25°C, 350 rpm.
6. After incubation aspirate the contents of the wells and wash the wells 5 times 300 µl Washing Buffer WB / well.
7. Following the last washing step pipette 100 µl of the diluted Antibody Conjugate in each well.
8. Cover the wells with sealing tape and incubate the plate for 1 hour at 20-25°C, 350 rpm.
9. After incubation wash the wells 5 times with Washing Buffer WB as described in step 6.
10. Following the last washing step pipette 100 µl of the diluted Enzyme Conjugate in each well.
11. Cover the wells with sealing tape and incubate the plate for 30 minutes at 20-25°C, 350 rpm.
12. After incubation wash the wells 5 times with Washing Buffer WB as described in step 6.
13. Pipette 100 µl of the Substrate Solution S in each well.
14. Incubate the microtiter plate for 30 minutes in the dark at room temperature (20°C -25°C).
15. Stop the reaction by adding 100 µl Stopping Solution STP to all wells.
16. Measure the absorbance within 30 minutes at 450 nm (Reference filter ≥ 590 nm).

Quality Control

Good laboratory practice (GLP) requires that controls are included in each assay. The test results are only

valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP or other applicable federal, state or local standards/laws. All Calibrators and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated.

Quality criteria

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance of Calibrator E should be above 1.0. Samples, which yield higher absorbance values than Calibrator E, should be re-tested with a higher dilution.

Calculation

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 samples, controls and calibrators.
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 concentrations of the diluted samples or the diluted control sera in ng/mL (or pg/mL according the chosen unit for the calibrators) are calculated in this way, the Resistin concentrations of the undiluted samples and of controls are calculated by multiplication with the respective dilution factor.

Exemplary calculation of Resistin concentrations

Sample dilution: 1:21

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:

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

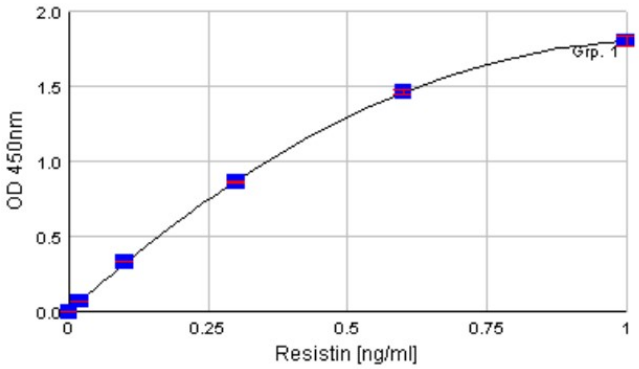
$$x = 0.2686$$

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

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

Typical Standard Curve

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



Precision

The inter- and intra assay coefficients of variability are in average < 10%.

Inter-Assay-Variation

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean	2.91	4.58	4.60	2.50	4.09
SD	0.16	0.24	0.23	0.09	0.27
CV [%]	5.55	5.33	5.04	3.37	6.67
n	26	15	17	7	23

Intra-Assay-Variation

	Sample 1	Sample 2
Mean [ng/mL]	2.81	4.79
SD	0.13	0.24
CV [%]	4.49	4.97
n	16	15

Sensitivity

The analytical sensitivity was determined by measuring the blank value. For this purpose, the signal intensity of blank value + 2SD was converted into a resistin concentration using the calibration curve. The analytical sensitivity of the assay is 0.012 ng / mL.

Specificity

The cross-reactivities of several commercially available animal sera were tested in this assay. Commercially available sera from cattle, cat, chicken, dog, donkey, goat, guinea pig, horse, mouse, pig, rabbit, rat and sheep were diluted 1:10 and used in the test and the signal intensity was measured. No cross-reactivity could be detected.

Recovery

Different human sera were spiked with recombinant human Resistin in varying concentrations. The recovery

of Resistin yielded on average 98 % of the theoretically expected amount.

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

Interferences

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. None of the tested substances interfered significantly with Resistin measurement.

	Triglyceride (100 mg/mL)	Bilirubin (100 µg/mL)	Hemoglobin (1 mg/mL)
Sample 1	101	93	94
Sample 2	115	99	99
Sample 3	104	103	147

Effects of coagulation inhibitors were investigating by adding indicated amounts of inhibitors to SB enriched with 0.3 ng/mL Resistin. Relative amounts of Resistin measured in inhibitor containing samples in comparison to 0.3 ng/mL Resistin containing Sample Buffer SB are shown.

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

Precautions

1. For research and professional use only. The Creative Diagnostics kit is suitable only for in vitro use and not for internal use in humans and animals.
2. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Creative Diagnostics will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.
3. Do not use obviously damaged or microbial contaminated or spilled material.
4. This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.
5. Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.

6. Following components contain human serum: Controls CTR1 and CTR2

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