Human Resistin ELISA Kit

Enzyme Immunoassay for the quantitative determination of human Resistin in serum, plasma, cell culture supernatants, breast milk, saliva and urine

Catalog number: ARG80885

distributed in the US/Canada by:
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For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Resistin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Resistin present is bound by the immobilized antibody. After washing away any unbound substances, a Biotin-Resistin antibody conjugate is added to each well and incubate. After washing, HRP-Streptavidin is added and incubate. Then washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Renin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Resistin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
100X Biotin-Resistin antibody conjugate	120 µl	4°C
100X HRP-Streptavidin Conjugate	120 µl	4°C
Standards A-E (20, 100, 300, 600, 1000 pg/ml)	6 vials	4°C, lyophilized
Control 1&2	2 vials	4°C, lyophilized
Sample buffer	120 ml	4°C
Dilution buffer	25 ml	4°C
20X Wash buffer	50 ml	4°C

Substrate (TMB)	12 ml	4°C
Stop solution	12 ml	4°C
Sealing tape	3	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the Biotin-antibody and HRP-Streptavidin conjugate before use.
- If crystals are observed in the 20X Wash buffer, Sample buffer or Dilution buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Haemolytic samples appear to show falsely high Resistin levels, so haemolytic samples should be avoided.

Other samples - Aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X wash buffer with distilled water to yield 1X wash buffer.
- Controls: Reconstitute the lyophilized content with 250 µL Dilution buffer. The dilutions of the Control 1 and 2 in Sample Buffer should be according to the dilution of the respective samples.
- Biotin-Resistin antibody conjugate: Dilute 1:100 with Dilution buffer.

- HRP-Streptavidin conjugate: Dilute 1:100 with Dilution buffer.
- Standards: Reconstitute the lyophilized contents of the standard vial with 750 µL Sample buffer.
- Sample: The samples have to dilute 1:10-50 fold with sample buffer. For clinical purposes we recommend a standard dilution of 1:20.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl Sample Buffer into blank wells.
3. Add 100 µl standards, controls and samples into each well.
4. Cover wells and incubate for 2 hours at RT.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl Biotin-antibody conjugate into each well. Incubate for 60 minutes at RT.
7. Aspirate and wash wells as step 5.
8. Add 100 µl of HRP-Streptavidin conjugate into each well. Incubate for 30 minutes at RT.

9. Aspirate and wash wells as step 5.
10. Add 100 µl of TMB Substrate to each well. Incubate for 30 minutes at room temperature in dark.
11. Add 100 µl Stop solution into each well.
12. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

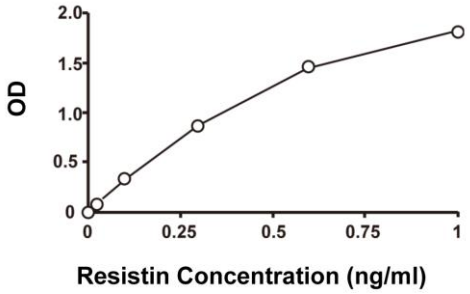
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimal detectable concentration is approximately 0.012 ng/ml.

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.

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 4.73% and the CV value of inter-assay precision was 5.19%.

Recovery

92.5-103.4%

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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