

## RESISTIN ELISA

EU:  IVD CAN:  USA: For Research Use Only. Not for Use in Diagnostic Procedures.

REF: CAN-RSN-4000 Version: 1.0  
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### INTENDED USE

For the quantitative determination of Resistin in human serum by an enzyme immunoassay.

### PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for resistin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of resistin is conjugated to biotin. During the first step, resistin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue coloured product that is directly proportional to the amount of resistin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microtiter plate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of resistin in patient samples and controls can be directly read.

### CLINICAL APPLICATIONS

Resistin is a 12.5 kDa protein containing 108 amino acids. It is synthesised as a pre-peptide and its hydrophobic signal peptide is cleaved before secretion. Resistin circulates in human blood as a dimeric protein consisting of two 92 amino acid polypeptides that are linked by a disulfide bridge.

Resistin belongs to the resistin-like molecule (RELM) hormone family. The RELM family comprises RELM- $\alpha$ , RELM- $\beta$ , RELM- $\gamma$  and resistin. RELM- $\beta$  is related to resistin and is expressed in the colon. In rodents, resistin is produced by adipose tissue and is a significant regulator of glucose metabolism and insulin sensitivity. Hyperresistinemia in rodents causes insulin resistance and predisposition to type 2 diabetes. In humans, resistin is produced by the macrophages, which stimulates the macrophage secretion of pro-inflammatory cytokines. Some studies have shown a correlation between increased serum resistin levels and atherosclerosis. Another study shows an increase of resistin levels in mice with atherosclerotic lesions. Many studies have tried to translate the mouse data to humans by answering the question whether levels of resistin are increased in human obesity, insulin resistance, and/or type 2 diabetes. Some groups failed to identify changes in resistin levels with obesity, insulin resistance, or type 2 diabetes while other studies that used diverse populations and different assays, have found significant relationships with one or more of these conditions.

Based on the above studies resistin may be a biomarker and

a mediator of metabolic and inflammatory diseases. Many areas of resistin physiology remain to be investigated to determine if it can be used as a marker for energy metabolism and body weight regulation, metabolic syndrome, inflammation and atherosclerosis.

### PROCEDURAL CAUTIONS AND WARNINGS

- This kit is intended for in vitro use only.
- Practice good laboratory practices when handling kit reagents. This includes:
  - Do not pipette by mouth.
  - Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
  - Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
  - Wash hands thoroughly after performing the test.
  - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Avoid microbial contamination of reagents.
- A calibrator curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (included in kit) should be included in every run and fall within the acceptable ranges, as stated in the quality control certificate.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
- When reading the microplate, the presence of bubbles in the microplate wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
- The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

### LIMITATIONS

- All the reagents within the kit are calibrated for the direct determination of resistin in human serum. The kit is not calibrated for the determination of resistin in other specimens of human or animal origin.

- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

### SAFETY CAUTIONS AND WARNINGS POTENTIAL BIOHAZARDOUS MATERIAL

All kit reagents and human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

### CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

### SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 2–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

### REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipette to deliver 50, 100, 150  $\mu$ L
- Disposable pipette tips
- Distilled or deionized water
- Orbital microplate shaker (capable of 600 rpm)
- Microplate washer (recommended)
- Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater

### REAGENTS PROVIDED

- Anti-Resistin Monoclonal Antibody Coated Break-Apart Well Microplate** — Ready To Use
  - Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
  - Storage: Refrigerate at 2–8°C
  - Stability: 12 months or as indicated on label.
- Monoclonal Anti-Resistin-Biotin Conjugate** — Ready To Use
  - Contents: One bottle containing a monoclonal anti-resistin antibody conjugated to biotin in a protein-based buffer with a non-mercury preservative.
  - Volume: 13 mL/bottle
  - Storage: Refrigerate at 2–8°C
  - Stability: 12 months or as indicated on label.

### 3. Streptavidin-Horseradish Peroxidase (HRP) Conjugate — Ready to Use

Contents: One bottle containing Streptavidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 13 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months in an unopened bottle or as indicated on label.

### 4. Resistin Calibrators — Ready to Use

Contents: Six vials containing resistin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of resistin. Calibrator concentrations\*: 0, 2, 5, 10, 15 and 20 ng/mL.

\* Approximate value - please refer to vial labels for exact concentrations.

Volume: Calibrators A–F: 1 mL/vial

Storage: Refrigerate at 2–8°C

Stability: 12 months in unopened vials or as indicated on label.

### 5. Resistin Controls — Ready to Use

Contents: Two vials containing resistin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of resistin.

Volume: 1 mL/vial

Storage: Refrigerate at 2–8°C

Stability: 12 months in unopened vials or as indicated on label.

### 6. Wash Buffer Concentrate — Requires Preparation $\times 10$

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

### 7. TMB Substrate — Ready To Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

### 8. Stopping Solution — Ready To Use

Contents: One bottle containing 1M sulfuric acid.

Volume: 6 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

## ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

- After all kit components have reached room temperature, mix gently by inversion. Prepare working solution of the wash buffer (see wash buffer concentrate under reagents provided section).
- Remove the required number of strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
- Pipette 50 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
- Pipette 100 µL of the monoclonal anti-resistin-biotin conjugate into each well (the use of a multichannel pipette is recommended).
- Incubate on a plate shaker (600 rpm on an orbital shaker) for 60 minutes at room temperature.
- Wash the wells **3 times** each time with 300 µL/well of working wash buffer solution. After washing, tap the plate firmly against absorbent paper to remove any residual liquid (the use of an automatic strip washer is strongly recommended). The performance of this assay is markedly influenced by the correct execution of the washing procedure.
- Pipette 100 µL of the streptavidin-HRP conjugate into each well (the use of a multichannel pipette is recommended).
- Incubate on a plate shaker (600 rpm on an orbital shaker) for 30 minutes at room temperature.
- Wash the wells **3 times** using the same procedure as in step 6.
- Pipette 150 µL of the TMB substrate into each well at timed intervals (the use of a multichannel pipette is recommended).
- Incubate on a plate shaker (600 rpm on an orbital shaker) for 10–15 minutes at room temperature or until calibrator F attains dark blue colour for desired OD.
- Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 10. Mix thoroughly by gently tapping the plate.
- Measure the absorbance at 450 nm in all wells with a microplate reader, within 20 minutes after addition of the stopping solution.

## CALCULATIONS

- Calculate the mean optical density of each calibrator duplicate.
- Plot a calibrator curve with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is used, choose a **4-parameter** or **5-parameter** curve fitting method.
- Calculate the mean optical density of each unknown duplicate.
- Read the values of the serum samples directly off the calibrator curve.

- If a sample reads more than calibrator F then dilute the original sample with calibrator A at a dilution of no more than 1:4. The result obtained must be multiplied by the dilution factor.

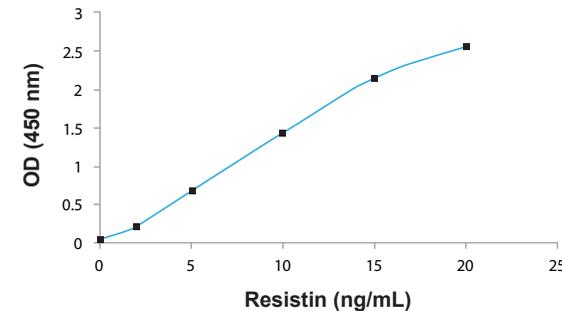
## TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Resistin (ng/mL)	Mean OD (450 nm)
A	0	0.044
B	2	0.204
C	5	0.571
D	10	1.221
E	15	1.846
F	20	2.207
Unknown	4.71	0.524

## TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



## PERFORMANCE CHARACTERISTICS

### SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 60 samples of the blank and calculated as follows:  
LoD = 2xSD

where SD is the standard deviation of the blank.  
**LoD = 0.004 ng/mL of Resistin.**

### SPECIFICITY (CROSS-REACTIVITY)

The blank was spiked separately with 100 ng/mL of human leptin, human TNF-α, human IL-6, human FABP4, human FABP5, human RELM-β or with 10 ng/mL of human C-peptide or 10 µg/mL of human adiponectin. The signal obtained for each was compared to the signal of resistin at 15 ng/mL.

% Cross-Reactivity = (Signal of substance tested / Signal of Resistin at 15 ng/mL) x100.

Analyte	Concentration (ng/mL)	%Cross-Reactivity
Leptin	100	0
TNF-α	100	0.21
IL-6	100	0
C-peptide	10	0
Adiponectin	10000	0
FABP4	100	0
FABP5	100	0
RELM-β	100	0

## INTERFERENCE

Interference testing was performed according to CLSI guideline EP7-A2. Serum samples with varying levels of resistin were spiked with potential interfering substances at recommended levels and analyzed. Results were compared to the same serum samples with no extra substances added to calculate the % interference. The following substances were tested and did not show significant interference in the assay: hemoglobin up to 0.25 g/L, bilirubin conjugated and free up to 85 µM, triglycerides up to 5.5 mg/mL and human serum albumin up to 60 g/L.

## RECOVERY

Three patient serum samples were spiked by adding defined amounts of resistin. The results are tabulated below:

Sample	Obs. Result (ng/mL)	Exp. Result (ng/mL)	Recovery %
1 - Unspiked + 2 ng/mL + 5 ng/mL + 10 ng/mL	6.36	-	-
	7.25	8.36	87
	9.70	11.36	85
	14.42	16.36	88
2 - Unspiked + 2 ng/mL + 5 ng/mL + 10 ng/mL	7.98	-	-
	8.96	9.98	90
	11.63	12.98	90
	16.71	17.98	93
3 - Unspiked + 2 ng/mL + 5 ng/mL + 10 ng/mL	10.36	-	-
	10.71	12.36	87
	13.69	15.36	89
	19.02	20.36	93

## LINEARITY

Three patient serum samples were diluted with calibrator A. The results are tabulated below.

Sample	Obs. Result (ng/mL)	Exp. Result (ng/mL)	Recovery %
Sample 1 1:2 1:4 1:8	14.53	-	-
	7.72	7.26	106
	4.10	3.63	113
	2.26	1.82	124
Sample 2 1:2 1:4	8.55	-	-
	4.71	4.27	110
	2.55	2.14	119
Sample 3 1:2 1:4	6.57	-	-
	3.6	3.28	110
	1.96	1.64	120

## INTRA-ASSAY PRECISION

Three samples were assayed 20 times each on the same calibrator curve. The results are tabulated below.

Sample	Mean (ng/mL)	SD (ng/mL)	CV%
1	3.61	0.068	1.9
2	8.32	0.17	2.0
3	15.79	0.44	2.8

## INTER-ASSAY PRECISION

Three samples were assayed in 20 different tests in the span of 20 days. The results are tabulated below.

Sample	Mean (ng/mL)	SD (ng/mL)	CV%
1	5.79	0.27	4.7
2	10.92	0.43	3.9
3	15.37	0.69	4.5

## COMPARITIVE STUDIES

The DBC Resistin ELISA kit (y) was compared with a leading competitor ELISA kit (x). The comparison of 40 serum samples yielded the following linear regression results:  
y = 0.9444 x - 0.2202 with r = 0.93.

## REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of reference values.

Group	n	Mean (ng/mL)	95% Confidence Range (ng/mL)
Male	120	4.28	1.53–8.95
Female	120	4.87	1.59–10.83

## REFERENCES

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## SYMBOLS

