CHEMICON® INTERNATIONAL
a Serologicals® Company

Human Resistin ELISA Kit

Cat. No. CYT352

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Obesity is a well-known risk factor of type 2 diabetes mellitus and is strongly associated with insulin resistance. Resistin (also called FIZZ3/ADSF) is an adipocyte-derived peptide first identified during a search for targets of thiazolidinediones. Steppan *et al*. reported that serum concentrations of resistin are markedly increased in obese mice and are decreased by treatment with thiazolidinediones (1). It was also found that administration of an antiresistin antibody increases insulin-stimulated glucose uptake in obese mice and that treatment of normal mice with recombinant resistin impairs insulin action. Thus, resistin might link obesity with insulin resistance and diabetes in mice models. However, subsequent studies in rodent models (2-4) have produced disparate findings on the role of resistin in obesity and insulin resistance. In humans, while the expression of resistin in human adipocytes is very low compared with that seen in rodents and does not differ between normal, insulin-resistant or type 2 diabetic individuals, a more recent study using a large size of case suggests that the plasma resistin levels are increased in type 2 diabetes (5-8). Genetic case-control studies have demonstrated that genetic variations in the resistin gene are associated with insulin resistance and obesity (9-10). More recently it has been shown that resistin acts on liver and antagonizes insulin signaling, thereby increasing gluconeogenesis and hepatic glucose output (11). This is the first study showing of the role of resistin in modulating physiological glucose metabolism. Therefore determination of the plasma resistin levels may be important for understanding onsets of metabolic diseases such as type 2 diabetes or obesity.

Test Principle

This kit is enzyme-linked immunosorbent assay (ELISA) for quantitative determination of Resistin in human serum.

Monoclonal antibody specific for human Resistin has been precoated onto 96-well microplate. Standards and samples are pipetted into the wells and any resistin present is bound by immobilized antibody. Bound resistin is captured by biotinylated anti-human resistin polyclonal antibody. HRP conjugated streptavidin is added. After washing, a substrate solution is added. The colors develop in proportion to the bound resistin quantity. The color development is stopped and the intensity of color is measured.

Application

The CHEMICON® Human Resistin ELISA Kit is designed to measure the amount of resistin in serum samples of human origin. There are enough reagents included in this kit for one 96-well immunoassay plate. Running duplicate wells for samples and standards is recommended.

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Analytical Sensitivity and Detection Limits

Sensitivity:	100 pg/mL.
Intra-assay Variation:	± 5.17 % (19.23 μg/mL)
Inter-assay Variation:	± 7.20 % (6.80 μg/mL)
Recovery:	92 ~108% for spiked samples
Cross-reactivity:	No cross-reactivity with mouse and rat sera. No reactivity detected with 30 ng/mL of mouse or rat resistin. No reactivity detected with 100 ng/mL of human adiponectin, RELM-β, Leptin, rat RELM-α, or mouse RELM-α or RELM-β.

Kit Components

1. 96-Well Plate: (Catalog No. CYT352a) 12x8 well strips coated with absorbed monoclonal antibody against human resistin.
2. Wash Concentrate: (Catalog No. CYT352b) One 100 mL (5X) bottle.
3. Assay Diluent: (Catalog No. CYT352c) One 50 mL (5X) bottle.
4. Secondary Antibody: (Catalog No. CYT352d) Anti-Human Resistin Biotinylated Polyclonal Antibody, 12 mL.
5. QC Sample: (Catalog No. CYT352e) Positive control, human serum (see vial label for value).
6. Detector: (Catalog No. CYT352f) One 150 μL (100X) bottle of HRP conjugated Streptavidin.

7. Resistin Standard (Recombinant Human): (Catalog No. CYT352g) One 16.0 ng/mL vial (Lyophilized).
8. Substrate I: (Catalog No. CYT352h) One 6 mL bottle.
9. Substrate II: (Catalog No. CYT352i) One 6 mL bottle.
10. Stop Solution: (Catalog No. CYT352j) One 12 mL bottle.

Materials Not Supplied

1. Precision single and multi-channel pipettes.
2. Disposable pipette tips.
3. Microtubes or equivalent for preparing dilutions.
4. Disposable plastic containers for preparing working detector antibody and substrate.
5. Reagent reservoirs.
6. Microwell or microstrip plate reader 450 nm.
7. Deionized water.

Precautions

- *Human Source Material*: The human source material supplied has been tested by an FDA approved method and found to be non-reactive for HIV-1/2 Antibody, HCV Antibody, a Serologic Test for Syphilis (STS), and Hepatitis B Surface Antigen (HBsAg). However, all materials should be handled carefully in accordance with good laboratory practices.
- The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and the failure to produce accurate data.

Preparation of Reagents

1. Allow all samples and kit components to equilibrate to room temperature (20° to 25°C).
2. Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use (See table below). It is recommended that standards and samples be run in duplicate.

3. **Wash Solution (1X)**

   Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.

4. **Diluent (1X)**

   Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).

5. **Detector (1X)**

   Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.

6. **Substrate Solution**

   Freshly prepare just before use the by adding one part Substrate I to one part Substrate II.

7. **Resistin Standard**

   Prepare working aliquots of the Standard as follows:

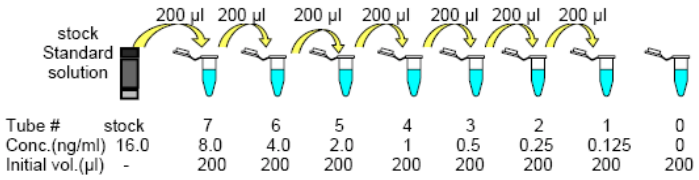
   Briefly centrifuge the Standard vial. When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water to the Standard vial to make a stock concentration of 16 ng/mL. Mix well.

   A recommended dilution scheme is as follows:

   a. Label 8 microcentrifuge tubes #0-7. Add 200 µL of the 1X Diluent to the microcentrifuge tubes # 0-7.

   b. Add 200 µL of the stock Standard solution to tube # 7 and vortex. This is Standard tube # 7 with a concentration of 8 ng/mL.

   c. Standards # 6 to 1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube # 0.



8. **QC Sample**
   Reconstitute QC sample in 1 mL of deionized water.

Serum Collection and Storage

Blood samples for measurement of serum resistin are collected in vacutainer tube and all tubes are centrifuged at 4°C for collection of serum. Other suitable samples include heparinated plasma, EDTA-treated plasma and citrate-treated plasma. These are stored at -80°C until analyses.

Storage

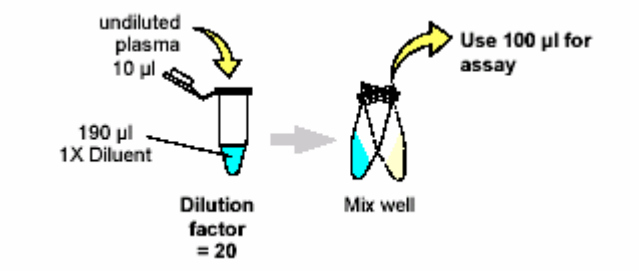
Reagents must be stored at 2° to 8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Preparation of Samples

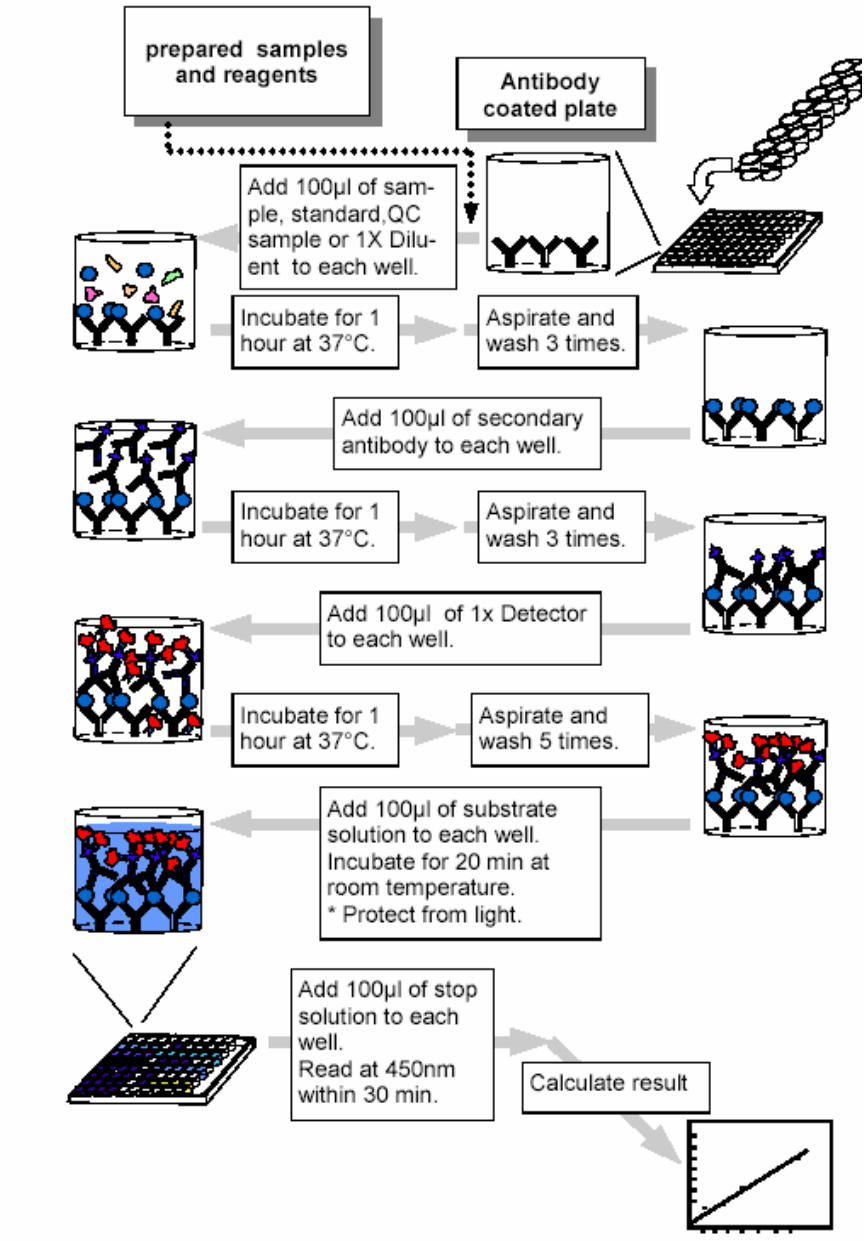
1. Dilute serum 1:20 with 1X Diluent (example, 10 µL serum plus 190 µL 1X Diluent; dilution factor=20) and mix well.
2. Use 100 µl of the final diluted serum for ELISA.

* If samples fall the outside range of assay, a lower or higher dilution may be required.

Do not dilute the QC Sample included in the kit.



Flow Chart of Assay Procedure

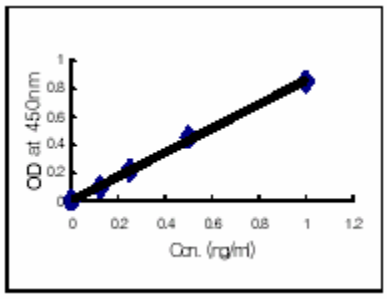


Assay Instructions

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 µL of standard 0 to 7, the reconstituted QC sample and diluted serum sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
3. Incubate at 37°C for 1 hour.
4. Remove the solution and wash 3 times with 250 µL of 1X Wash Solution to each well.
5. Add 100 µL Secondary Antibody to each well.
6. Incubate at 37°C for 1 hour.
7. Remove the solution and wash 3 times with 250 µL of 1X Wash Solution to each well.
8. Add 100 µL 1X Detector to each well.
9. Incubate at 37°C for 1 hour.
10. Remove the solution and wash 5 times with 250 µL of 1X Wash Solution to each well.
11. Add 100 µL of the Substrate Solution to each well.
12. Incubate at room temperature for 20 min. * Protect from light.
13. Using the multi-channel pipette, add 100 µL Stop Solution to each well.
14. Read at 450 nm.

Calculation of Results

1. Subtract the absorbance of the blank from the readings for each standard and sample.
2. Construct the standard curve by plotting the known concentration (X) of standard versus the absorbance (Y) of standard. A typical linear range is between 0.125 ng/mL and 1 ng/mL.



Sample Graph

3. Calculate the resistin concentrations of samples by interpolation of the regression curve formula.
4. The resistin concentrations calculated for the unknown samples and QC sample must be multiplied by the dilution factor [see **Preparation of Samples**] to obtain the concentrations of the undiluted samples.

References

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Troubleshooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order
	Washes too stringent	Use an automated plate washer if possible
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use
High background	Concentration of detector too high	Use recommended dilution factor
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

Warranty

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