Human Resistin ELISA PROCEDURE SUMMARY

Total Assay Time - 195 min. (90+60+30+15)

	Allow all reagents to reach room temp. arrange and label required # of strips. Dilute sample Diluent (1:20) and wash buffer (1:100) with water. Dilute serum samples (0-1:10), Detection antibody (1:100), and HRP-conjugate (1:100) with 1x sample diluent. Do not dilute standards.
Step 1	Pipet **20 ul** of pre-diluted **standards** and diluted samples (no dilution-1:10) into appropriate wells. Add 80ul of 1x sample diluent. Mix gently, cover the plate and incubate for **60 min** at room temp
Step 2	Aspirate and **wash 3 times** with 1x wash solution**.** Dispense **100 ul of 1x Biotinylated Detection antibody** to each well. Mix gently, cover the plate and incubate for **60 min** at room temp.
Step 3	Aspirate and **wash 3 times** with 1x wash solution**.** Dispense **100 ul of 1x HRP-streptavidin-conjugate** to each well. Mix gently, cover the plate and incubate for **30 min** at room temp.
Step 4	Aspirate and **wash 3 times** with 1x wash solution. Dispense **100 ul of TMB substrate Solution**. Mix gently, cover the plate and incubate for **15 min** at room temp. Blue color develops
Step 5	Pipette 100 ul stop solution (1N H2SO4) into each well. Blue color turns yellow. Measure Absorbance at 450 nm.

CHECK LIST (Check each box after completing each of the above steps)

	Step 1	Step2	Step3	Step4	Step5
Start time End Time					

KIT PROFILE

Date received: **Cat #** 100-110-RSH **Lot #** _____ **Exp.** _____

Date kit opened _____ **Technician:** _____

Date used: **# Strips used** _ **# Remaining** _____

Date used: **# Strips used** _ **# Remaining** _____

Remarks _____

_____

Instruction Manual No. M-100-110-RSH

Human Serum Resistin

ELISA Kit Cat. # 100-110-RSH

For Quantitative Determination of Resistin In Human Serum

Human Resistin ELISA KIT Cat. No. 100-110-RSH

Kit Components, 96 tests	Cat #
Anti-Human Resistin coated strip plate (8 wells x 12 strips)	100-111
Human Resistin Std. A (0 ng/ml), 0.250 ml	100-112
Human Resistin Std B (0.25 ng/ml), 0.250 ml	100-113
Human Resistin Std C (0.5 ng/ml), 0. 250 ml	100-114
Human Resistin Std D (1 ng/ml), 0. 250 ml	100-115
Human Resistin Std E (1.5 ng/ml), 0. 250 ml	100-116
Human Resistin Std F (2 ng/ml), 0. 250 ml	100-117
(20x) Sample/Conjugate Diluent, 10ml	SD-20
Wash Buffer (100X), 10 ml	WB-100
(100x) Anti-Human Resistin Detection Ab, 0.12 ml	100-118
(100x) Streptavidin-HRP conjugate, 0.12ml	S-HRP100
TMB Substrate, 12 ml	80091
Stop solution, 12 ml	80101
Instruction Manual	M-100-110-RSH

Introduction

Obesity, a common nutritional disorder, is associated with diabetes, hypertension, hyperlipidemia, cancer and many other health related problems. Regulation of body weight depends upon the calorie intake and expenditure. It is a very complex and highly regulated process. It involves multiples neural circuits with specific neuropeptides, neurotransmitter transporters and receptors and influenced by peripheral signals. Resistance to insulin characterizes type 2 diabetes, the most common form of diabetes. There is a strong link between type 2 diabetes and obesity, as most patients tend to be obese. Adipose tissue is the major organ involved in energy storage and mobilization. Adipocyte produces several key regulatory proteins such as leptin, adipsin, Acrp30/AdipoQ, TNF-alpha, adipocyte fatty acid binding protein (aFABP) and Pref-1 (an inhibitor or adipocyte differentiation) that are involved in obesity. Thiozolidinediones (TZDs), a new class of anti-diabetic, enhances target-tissue sensitivity to insulin.

A screen of genes down regulated by TZD in adipocyte led to the discovery of a new protein hormone called resistin (for resistance to insulin). Resistin, specifically produced and secreted by adipocyte, is present at elevated levels in the blood of obese animals, and is down regulated by fasting and anti-diabetic drugs. Antibody to resistin stimulated glucose uptake and improved insulin sensitivity in obese mice. Some other protein related to resistin has been called resistin-related molecules (RELM-alpha and beta). Resistin family of proteins was also identified as proteins (FIZZ1-3, for Found in Inflammatory zone) involved in allegory and inflammation.

ADI Resistin ELISA kit is a highly sensitive sandwich type assay for the measurement of Resistin in serum. The assay can be adapted to measure Human Resistin in other biological fluids such as plasma, urine, culture medium etc.

PERFORMANCE CHARACTERISTICS

1. Detection limit- Based on 6 replicate determinations of the zero standards, the minimum Resistin concentration detectable using this assay is 200 pg/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. High dose hook effect

Resistin concentrations of up to 20 ng/ml did not show any hook effect.

3. Expected Values: A limited testing of 20 adult Human serum samples values of 4.65 - 16.30 ng/ml (average 9.83 ng/ml).

4. Specificity: This kit is specific to Resistin and does not show any significant reactivity to other Human serum proteins.

5. Species Crossreactivity

Cross reactivity was tested with the following animal serum at no dilution: Monkey serum cross-reacted at 38%. Rat, Goat, Mouse, and Hamster serums had less than 1% crossreactivity.

Other ELISA kits are available from ADI (complete list at the web site)

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).
Monkey: IgM, IgG, IgA, CRP
Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein
Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF,
Chicken: IgG, IgM, IgY, Ovalbumin
Rabbit: CRP, IgG
Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin
Pig: Albumin, IgG, IgM,
Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG
Sheep: IgG
Turkey: IgG

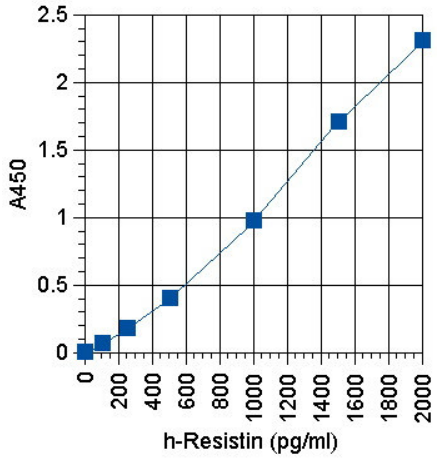
For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	*Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Std. A** (0 ng/ml)	0.000	
B1, B2	**Std. B** (0.25 ng/ml)	0.21	
C1, C2	**Std. C** (0.5 ng/ml)	0.43	
D1, D2	**Std. D** (1 ng/ml	0.998	
E1, E2	**Std. E** (1.5 ng/ml)	1.73	
F1, F2	**Std. f** (2 ng/ml)	2.35	
G1, G2	**Sample 1 (1:5)**	0.561	(2.01 ng/ml) Adjusted for sample dilution 10.05 ng/ml

*=Average duplicate values after deducting the std zero values.

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Human Resistin ELISA kit is based on binding of Human Resistin from samples to two antibodies, one immobilized on the microtiter well plates, and other bound to the enhancing protein Biotin, which then binds to strepavidin horseradish peroxidase conjugate. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of Resistin present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of Resistin in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000ul) and multi-channel pipette with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Alpha Diagnostic International Human Resistin ELISA kit is intended for *in vitro research* use only. The reagents contain Thimerosal (0.01%) as preservative; necessary care should be taken when disposing solutions. All other precautions must be taken to handle biological material.

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid) and dispose of it accordingly.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20^{O}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. It is also possible to use plasma for testing.

Reagent Preparation

1. Dilute the 20x Sample Diluent 1:20 with water (5ml diluent in 95ml water). Dilute only the required reagent. Store diluted solution at 4oC for 3-4 days. Prepare 100ml for a full plate assay.

2. Dilute the wash buffer 1:100 with water. Dilute 5ml of the stock in 500ml water. Store at room temperature for 1 week.

3. Dilute the Detection Ab. (1:100) using the 1x Sample Diluent. (100ul in 10ml diluent). Prepare 10ml for a full plate assay.

4. Dilute the Streptavidin HRP (1:100) using the 1x Sample Diluent (100ul in 10ml diluent). Prepare 10ml for a full plate assay.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

1. **Do not dilute standards**. Dilute Human serum samples 1:5 using 1x Sample Diluent. Some samples may have to be diluted more or less but 1:5 should bring most normal samples to within the testing range. Note: It is possible to adjust the sample dilution to make no dilution and a 1:10 dilution and we recommend testing them both.

2. Label or mark the microtiter well strips to be used on the plate.

3. Pipet **20ul stds**. and diluted samples into appropriate wells.

4. **Note:** for ease of loading samples it is recommended that a second **uncoated** microwell plate should be used keeping diluted samples. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipette.

5. Add **80ul** of 1x Sample Diluent to all wells. Mix gently, cover the plate and incubate at room temperature for **90 min**.

6. Wash the wells with **3 times with 300 ul** of 1x wash buffer.

7. **Pipette 100 ul** of 1x Resistin Detection antibody into each well. Mix gently. Cover the plate and incubate for **60 minutes** at room temperature. **Note:** the conjugate solution must be at room temperature.

8. Wash the wells with **3 times with 300 ul** of 1x wash buffer.

9. Pipette **100 ul of 1x Streptavidin-HRP-enzyme** into **each well. Mix gently.** Cover the plate and incubate for **30 minutes** at room temperature. **Note:** the conjugate solution must be at room temperature**.**

10. Aspirate and wash the wells **3 times** with 1x wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

11. Add **100 ul** of HRP-substrate soln. (TMB) into each well. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops. **Note:** TMB solution must be at room temperature.

12. Stop the reaction by adding **100 ul of stop** solution to **all wells**. Mix gently. Blue color turns yellow.

13. Measure the absorbance at **450 nm** using an ELISA reader. Color is stable for at least 30 min after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{O}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Samples containing more than **2 ng/ml** Human Resistin should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards and samples. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate Resistin concentrations. Read off the Resistin concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:5 then the values must be multiplied by 5 and results expressed as pg/ml.