Human Resistin ELISA Kit

Introduction

Resistin, a novel adipose-derived protein, has been proposed to cause insulin-resistant states in obesity (1). Resistin is produced by white and brown adipose tissues but has also has been identified in several other tissues, including the hypothalamus, pituitary and adrenal glands, pancreas, gastrointestinal tract, myocytes, spleen, white blood cells and plasma. Resistin antagonizes insulin action, and is down regulated by rosiglitazone and peroxisome proliferator-activated receptor gamma agonists (2). Resistin is elevated in patients with Type II diabetes and may play a role in the vascular complications of this disorder (3). Recently, resistin has been discussed controversially as a missing link between obesity and insulin resistance (4).

Principal of the Assay

The Human Resistin ELISA kit is designed for detection of human resistin in plasma, serum, urine, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique, which measures resistin in less than 5 hours. A murine monoclonal antibody specific for resistin has been pre-coated onto a microplate. Resistin standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for resistin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Resistin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against resistin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Resistin Standard :** Human resistin in a buffered protein base (16 ng, lyophilized).
- **Biotinylated resistin Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human resistin (80 µl).
- **EIA Diluent Concentrate (10x):** A 10-fold buffered protein base (30 ml).

- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Opened EIA Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:5 with EIA Diluent. Store the remaining samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:5 into EIA Diluent. Store serum at -20^{0}C or below. Avoid repeated freeze-thaw cycles
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Urine dilution is suggested at 1:8 into MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **EIA Diluent Concentrate (10x):** Dilute EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 16 ng of human Resistin Standard with 1 ml of EIA Diluent to generate a stock solution of 16 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points

by serially diluting the Resistin standard solution (16 ng/ml) twofold with equal volume of EIA Diluent to produce 8, 4, 2, 1, 0.5 and 0.25 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at < -20^{0}C (Store for no more than one week).

Standard Point	Dilution	[Resistin] (ng/ml)
P1	1 part Standard (16 ng/ml)	16.000
P2	1 part P1 + 1 part EIA Diluent	8.000
P3	1 part P2 + 1 part EIA Diluent	4.000
P4	1 part P3 + 1 part EIA Diluent	2.000
P5	1 part P4 + 1 part EIA Diluent	1.000
P6	1 part P5 + 1 part EIA Diluent	0.500
P7	1 part P6 + 1 part EIA Diluent	0.250
P8	EIA Diluent	0.000

- **Biotinylated Resistin Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at < -20^{0}C.
- **Wash Buffer Concentrate (20x):** Dilute Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at < -20^{0}C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
  If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated Resistin Antibody to each well and incubate for two hours.
- Wash a microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some

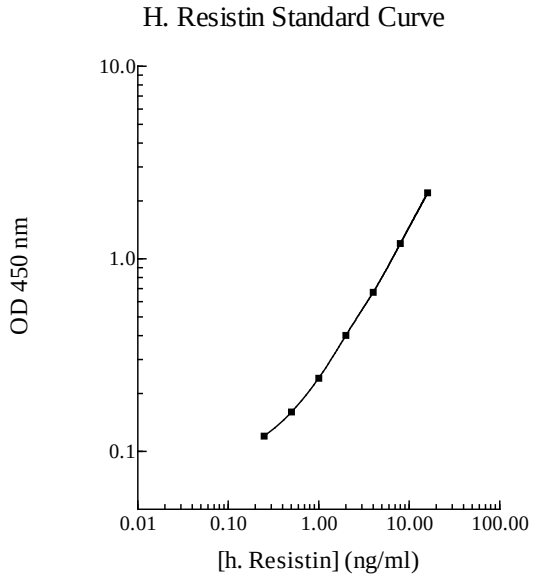
unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable level of Resistin is typically 0.2 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.2 % and 7.3% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:5	101%	99%
1:10	100%	102%
1:20	101%	98%

Recovery

Standard Added Value	0.5 – 5 ng/ml
Recovery %	84-111 %
Average Recovery %	99.5 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	90%
Bovine	1%
Monkey	90%
Mouse	None
Rat	None
Swine	5%
Rabbit	20%

- 10% FBS in culture media will not affect the assay.