ELISA Kit Components	Amount	Cat/Part No.
Anti-Resistin Microwell Strip Plate	8-well strips (12)	100-121
Resistin Standard, lyophilized	3 vials	100-122
Anti-Resistin Detecting Antibody (100X)	0.15 ml	100-123
Streptavidin HRP Conjugate (100X)	0.15 ml	S-HRP100
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	100-120-RSM

Other ELISA kits available from ADI

Human: BD-1, BD-2, BD-3 **and**: 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, IgG1, IgG4, IgA, Insulin, NSE, CA125, CA199, CA242, PAP, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgM, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Monkey: IgM, IgG, IgA, CRP
Chicken: IgG, IgM, IgY, Ovalbumin
Rabbit: CRP, IgG
Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG

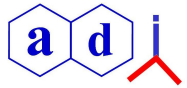
For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. 100-120-RSM

Mouse Resistin

ELISA Kit Cat. No. 100-120-RSM

For Quantitative Determination of Resistin in Solution



ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

INTENDED USE

The Mouse Resistin ELISA Kit is an in vitro immunoassay for research use in the quantification of resistin in cultures of mouse cells and in appropriately qualified samples from serum, saliva, or other tissue fluids.

RESEARCH USE OF THE TEST

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, and involves multiple neural circuits with specific neuropeptides, neurotransmitter transporters and receptors, 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. Adipocytes produce several key regulatory proteins such as leptin, adipsin, Acrp30/AdipoQ, TNF-alpha, adipocyte fatty acid binding protein (aFABP) and Pref-1 (an inhibitor of 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 proteins related to resistin has been called resistin-related molecules (RELM-alpha and beta). Resistin family of proteins was also identified as proteins FIZZ1-3 (Found in Inflammatory Zone) involved in allergy and inflammation.

PRINCIPLE OF THE TEST

The Mouse Resistin ELISA kit is based on the binding of mouse resistin in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to biotin, which then binds to a streptavidin horseradish peroxidase (HRP) conjugate. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of resistin present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of resistin in samples is calculated from a standard curve of purified recombinant mouse resistin of designated concentration.

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. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity
The antibodies used in this kit have been affinity purified using a purified recombinant mouse resistin immunosorbent and have been shown by ELISA to react specifically with mouse natural and recombinant resistin, partial reactivity with rat resistin and to have essentially no reactivity with recombinant human resistin. The antibodies show essentially no reactivity with other mouse proteins.

Accuracy
The standards of this assay have been calibrated against highly purified recombinant mouse resistin, expressed in E. coli.

Mouse Serum
Resistin Levels
Assay of stored, frozen sera from eleven (11) individual swiss mice and one serum pool ranged from 10 to 130 ng/ml. Normal value ranges should be established for the laboratory's expected testing populations.

Recovery
Purified resistin was spiked into each of 10 stored serum samples diluted 1:100. All samples showed good recovery, ranging from **91** to **108**% of expected values.

Culture Medium
Linearity of Dilution and Recovery
Mouse Resistin Standard (lyophilized), reconstituted and diluted with 10% Neonatal Bovine Serum, was essentially parallel with the normal Standard Curve. For samples from cultured cells, the standards should be diluted in the culture medium to produce linear dilution and equivalent quantification across the standard range.

CALCULATION OF RESULTS

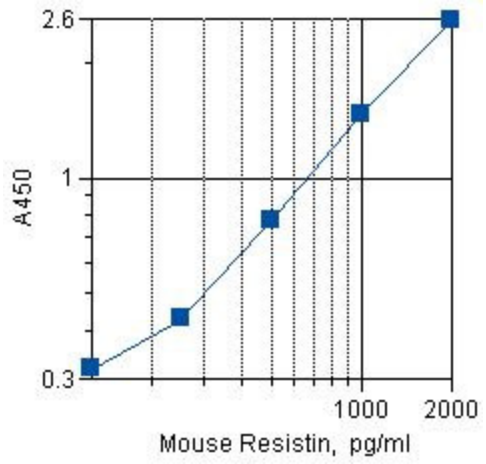
The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, resistin concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (pg/ml) of resistin (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The resistin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 2000 pg/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	Resistin pg/ml
A1, A2	**Negative Diluent Control**	0.15	0
B1, B2	125 pg/ml **Standard**	0.32	125
C1, C2	250 pg/ml **Standard**	0.43	250
D1, D2	500 pg/ml **Standard**	0.77	500
E1, E2	1000 pg/ml **Standard**	1.46	1000
F1, F2	2000 pg/ml **Standard**	2.57	2000
G1, G2	**Sample** [Diluted 1:5]	0.88	550
	Calculated: 5-fold dilution x 550 pg/ml = **2.75** ng/ml in serum		



To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Mouse Resistin Standard Part No. 100-122	Three (3) vials, each containing resistin lyophilized in buffer with protein, detergents and ProClin 300 as stabilizers. Keep lyophilized vials frozen until used or kit lot expires.

Reconstitute 1 vial with 0.50ml **Working Sample Diluent*** to provide a 2000 pg/ml Top Standard, sufficient for one entire curve. Prepare 2-fold dilutions, as follows:

Standard	+ Diluent	= Final Concn
Reconstituted Standard	None	2000 pg/ml
250ul ul of 2000pg/ml	250ul	1000 pg/ml
250ul ul of 1000pg/ml	250ul	500 pg/ml
250ul ul of 500pg/ml	250ul	250 pg/ml
250ul ul of 250pg/ml	250ul	125 pg/ml

Use within 2 weeks of preparation.

Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Mouse Resistin Detection Antibody Concentrate (100x) Part No. 100-123, 0.15ml	Biotinylated anti-mouse resistin in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.
Streptavidin-HRP Conjugate Concentrate (100x) Part No. S-HRP100, 0.15ml	Peroxidase conjugated streptavidin in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

- Please see **Culture Medium**, page 6.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Mouse Resistin Microwell Strip Plate	100-121	8-well strips (12)	Coated with purified anti-mouse resistin antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples, Detection Antibody Concentrate and Streptavidin-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent, Detection Antibody and Streptravidin-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, stored refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

Use freshly diluted Standards as described on page 2. Dilute samples in Working Sample Diluent according to expected resistin concentrations. Dilute serum and other body fluids at least 10-fold to avoid sample matrix issues; culture medium may be used neat.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation [100ul - 90min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 90 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul - 60min; 4 washes]

- Add 100ul of Working Detection Antibody to each well.
- Incubate for 60 minutes.
- Wash wells 4 times as in step 2.

4. 3rd Incubation [100ul - 30min; 5 washes]

- Add 100ul of Working Streptavidin-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

5. Substrate Incubation [100ul - 15min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

6. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

7. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.