DRG® Mouse Resistin ELISA (EIA-4283)

Revised 18 Apr. 2006 **For Veterinary Use Only**

INTENDED USE

This Mouse Resistin ELISA kit is used for the non-radioactive quantification of Mouse Resistin in mouse serum, plasma, adipocyte extracts or cell culture media samples. This kit has 100% cross reactivity to Mouse Resistin. One kit is sufficient to measure 38 unknown samples in duplicate. ***This kit is for research purpose only.***

PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) capture of Mouse Resistin molecules from samples to the wells of a microtiter plate coated by a pre-titered amount of anti-mouse resistin monoclonal antibodies, 2) wash away of unbound materials from samples, 3) binding of a second biotinylated anti-mouse polyclonal antibody to the captured molecules, 4) wash away of unbound materials from samples, 5) conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, 6) wash away of free enzyme conjugates, and 7) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured Mouse Resistin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Mouse Resistin.

REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

A. Mouse Resistin ELISA Plate
Coated with Monoclonal anti-Mouse Resistin Antibodies
Quantity: 1 plate
Preparation: Ready to Use

B. Adhesive Plate Sealer
Quantity: 2 sheet
Preparation: Ready to Use

A. 10X HRP Wash Buffer Concentrate
10X concentrate of 50mM Tris Buffered Saline containing Tween-20.
Quantity: 2 bottles containing 50 ml each
Preparation: Dilute 1:10 with distilled or deionized water.

B. Mouse Resistin Standard
Purified Recombinant Mouse Resistin, 50 ng lyophilized.
Quantity: 1ml upon hydration
Preparation: Contents Lyophilized. Reconstitute with 1 ml distilled or deionized water.

E. Mouse Resistin Quality Controls 1 and 2
Purified Recombinant Mouse Resistin, lyophilized.
Quantity: 1ml/vial upon hydration
Preparation: Contents Lyophilized. Reconstitute with 1 ml distilled or deionized water.

Revised 18 Apr. 2006 **For Veterinary Use Only**

A. Assay Buffer
Buffer containing BSA and 0.08% Sodium Azide
Quantity: 20 ml
Preparation: Ready to Use

B. Mouse Resistin Detection Antibody
Pre-titered Biotinylated Goat anti-Mouse Resistin Polyclonal Antibody
Quantity: 6 ml
Preparation: Ready to Use

C. Enzyme Solution
Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer
Quantity: 12 ml
Preparation: Ready to Use

I. Substrate (Light sensitive, avoid unnecessary exposure to light)
3, 3', 5, 5'-tetramethylbenzidine in buffer
Quantity: 12 ml
Preparation: Ready to Use.

A. Stop Solution (Caution: Corrosive Solution)
0.3 M HCl
Quantity: 12 ml
Preparation: Ready to Use

B. Matrix Solution
Quantity: 1 ml/vial
Preparation: Ready to Use

STORAGE AND STABILITY

Prior to use, all components in the kit can be stored up to 2 weeks at 2-8°C. For longer storage(> 2 weeks), freeze Wash Buffer, Assay Buffer, Serum Matrix, Resistin Standards, Quality Controls and reconstituted Standards and Controls at ≤ –20°C. Minimize repeated freeze and thaw of the Resistin Standards and Quality Controls. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

REAGENT PRECAUTIONS

A. Sodium Azide

Sodium azide has been added to certain reagents as a preservative. Although the concentrations are low, sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

B. Hydrochloric Acid

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and Pipette Tips: 10µl - 20 µl or 20µl - 100 µl
2. Multi-Channel Pipettes and Pipette Tips: 5 ~ 50 µl and 50 ~ 300 µl
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth

SAMPLE COLLECTION AND STORAGE

1. To prepare serum samples, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.
   Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 ± 2°C.
   Transfer and store serum samples in separate tubes. Date and identify each sample.
   Use freshly prepared serum or aliquot and store samples at ≤ –20°C for later use. For long-term storage, keep at -70 °C. Avoid freeze/thaw cycles.
2. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.735 mg/ml and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.
3. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
4. Avoid using samples with gross hemolysis or lipemia.

SAMPLE PREPARATION

1. No dilution or preparation is needed for normal serum or plasma samples. In the event that any sample is above 50 ng/ml range, dilutions should be performed using the Serum Matrix provided.

2. Adipocyte extracts or cell culture media samples may require dilution. Dilutions should be performed using the assay buffer provided.

STANDARD AND QUALITY CONTROLS PREPARATION

A. Mouse Resistin Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using an Eppendorf pipette, reconstitute the Mouse Resistin Standard with 1 ml distilled or deionized water into the glass vial to give a 50 ng/mL concentration of Standard. Invert and mix gently, let sit for 5 minutes then mix well.

2. Label six tubes 25, 12.5, 6.25, 3.125, 1.56, and 0.78ng/ml. Add 0.5 ml Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 0.5 ml of the 50 ng/ml reconstituted standard to the 25 ng/ml tube, mix well and transfer 0.5 ml of the 25 ng/ml standard to the 12.5 ng/ml tube, mix well and transfer 0.5 ml of the 12.5 ng/ml standard to the 6.25 ng/ml tube, mix well and transfer 0.5 ml of the 6.25 ng/ml standard to the 3.125 ng/ml tube, mix well and transfer 0.5 ml of the 3.125 ng/ml standard to the 1.56 tube, mix well and transfer 0.5 ml of the 1.56 ng/ml standard to the 0.78 ng/ml tube and mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Standard Concentration ng/ml	Volume of Deionized Water to Add	Volume of Standard to Add
50	1 ml	0

Standard Concentration ng/ml	Volume of Assay Buffer to Add	Volume of Standard to Add
25	0.5 ml	0.5 ml of 50 ng/ml
12.5	0.5 ml	0.5 ml of 25 ng/ml
6.25	0.5 ml	0.5 ml of 12.5 ng/ml
3.125	0.5 ml	0.5 ml of 6.25 ng/ml
1.56	0.5 ml	0.5 ml of 3.125 ng/ml
0.78	0.5 ml	0.5 ml of 1.56 ng/ml

B. Mouse Resistin Quality Control 1 and 2 Preparation

1. Use care in opening the lyophilized Quality Control vials. Using an Eppendorf pipette, reconstitute each of the Mouse Resistin Quality Control 1 and Quality Control 2 with 1 ml distilled or deionized water into the glass vials. Invert and mix gently, let sit for 5 minutes then mix well.

ASSAY PROCEDURE

Pre-warm all reagents to room temperature prior to setting up the assay.

1. Dilute the 10X concentrated Wash Buffer 10 fold by mixing the entire contents of both buffer bottles with 900 ml deionized or distilled water.

2. Remove the microtiter assay plate from the foil pouch and fill each well with 300 µl of diluted Wash Buffer. Incubate at room temperature for 5 minutes. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, use a gentle wash program for all washing steps described in this protocol.**

3. Add in duplicate, 50µl Assay Buffer to Standard wells, and QC1 and QC2 wells.
(See plate well map for suggested well orientation).

4. Add in duplicate, 60µl Assay Buffer to the blank wells and sample wells.

5. Add 10µl Matrix Solution to Standard wells, QC1 and QC2 wells, and blank wells.

6. Add in duplicate, 10 µl mouse Resistin Standards in the order of ascending concentration to the appropriate wells. Add in duplicate, 10 µl QC1 and 10 µl QC2 to the appropriate wells. Add sequentially, 10µl of the unknown samples in duplicate to the remaining wells. **For best result all additions should be completed within 30 minutes.**
(Note: Capture step is a total volume of 70µl and all subsequent steps are a total volume of 50µl/well)

7. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.

8. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

9. Wash wells 3 times with diluted Wash Buffer, 300 µl per well per wash. Decant and tap firmly after each wash to remove residual buffer.

10. Add 50 µl Detection Antibody to all wells. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.

11. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

12. Wash wells 3 times with diluted Wash Buffer, 300 µl per well per wash. Decant and tap firmly after each wash to remove residual buffer.

13. Add 50 µl Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

14. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.

15. Wash wells 3 times with diluted Wash Buffer, 300 µl per well per wash. Decant and tap firmly after each wash to remove residual buffer.

16. Add 50 µl of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for approximately 12 to 20 minutes (A longer development time may be needed if using a plate washer). Blue color should be formed in wells of Resistin standards with intensity proportional to increasing concentrations of Resistin.
    NOTE: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

1. Remove sealer and add 50 µl Stop Solution **[CAUTION: CORROSIVE SOLUTION]** and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Read absorbance at 450 nm and 590nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of the highest Resistin standard should be approximately 2.0-2.8, or not to exceed the capability of the plate reader used.

Assay Procedure for Mouse Resistin ELISA kit:

DRG® Mouse Resistin ELISA (EIA-4283)

Revised 18 Apr. 2006 **For Veterinary Use Only**

	Step 1	Step 2	Step 3-4	Step 5	Step 6	Step 7-9	Step 10	Step 11-12	Step 13	Step 14-15	Step 16	Step 16-17	Step 17	Step 17
Well #	Dilute both bottles of 10X Wash Buffer with 900ml Deionized Water.	Wash plate with 300 µl Wash Buffer and incubate at room temperature for 5 minutes. Remove residual buffer by tapping smartly on absorbent towels	Assay Buffer	Matrix	Standards/ Controls/ Samples	Seal, Agitate, Incubate 1 hour at Room Temperature. Wash 3X with 300 µl Wash Buffer	Detection Ab	Seal, Agitate, Incubate 1 hour at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels. Wash 3X with 300 µl Wash Buffer	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature . Wash 3X with 300 µl Wash Buffer	Substrate	Seal, Agitate, Incubate 12-18 minutes at Room Temperature.	Stop Solution	Read Absorbance at 450 nm and 590 nm.
A1, A2			60 µl	10µl	----------		50 µl		50 µl		50 µl		50 µl	
A3, A4			50 µl	10µl	10 µl of 0.78 ng/ml Standard									
A5, A6			50 µl	10µl	10 µl of 1.56 ng/ml Standard									
A7, A8			50 µl	10µl	10 µl of 3.125 ng/ml Standard									
A9, A10			50 µl	10µl	10 µl of 6.25 ng/ml Standard									
A11, A12			50 µl	10µl	10 µl of 12.5 ng/ml Standard									
B1, B2			50 µl	10µl	10 µl of 25 ng/ml Standard									
B3, B4			50 µl	10µl	10 µl of 50 ng/ml Standard									
B5, B6			50 µl	10µl	10 µl of QC I									
B7, B8			50 µl	10µl	10 µl of QC II									
B9, B10			60 µl	0	10 µl of Sample									
B11, B12			60 µl	0	10 µl of Sample									

MICROTITER PLATE ARRANGEMENT

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	0.78 ng/ml	0.78 ng/ml	1.56 ng/ml	1.56 ng/ml	3.125 ng/ml	3.125 ng/ml	6.25 ng/ml	6.25 ng/ml	12.5 ng/ml	12.5 ng/ml
B	25 ng/ml	25 ng/ml	50 ng/ml	50 ng/ml	QC 1	QC 1	QC 2	QC 2	Sample	Sample	Sample	Sample
C	Sample	Sample	Sample	Sample	Etc.							
D												
E												
F												
G												
H												

n.d. = not detectable

CALCULATIONS

The dose-response curve of this assay fits best to a 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5-parameter logistic function.

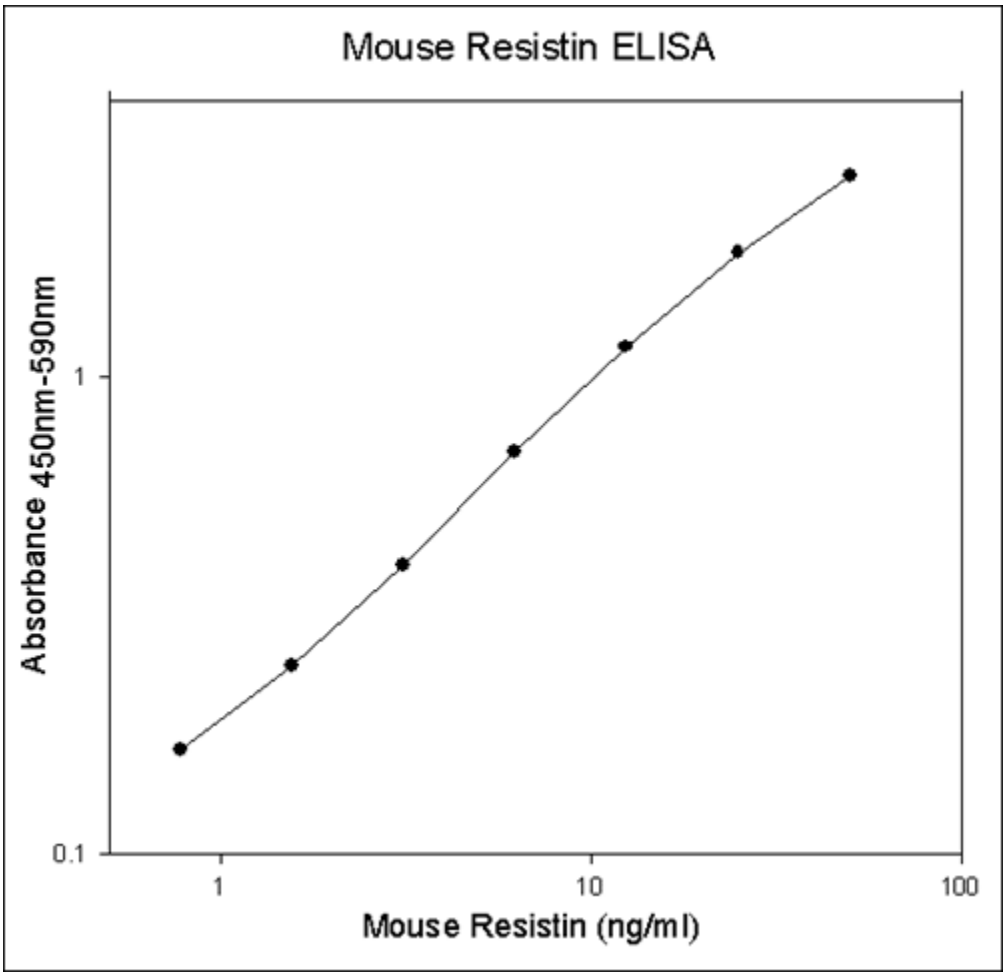
Note: When sample volumes assayed differ from 10 µl, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 5 µl of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10 µl, compensate the volume deficit with matrix solution.

INTERPRETATION

A. Acceptance Criteria

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.
3. The limit of sensitivity of this assay is 0.78 ng/ml mouse Resistin (10 µl sample size).
4. The appropriate range of this assay is 0.78 ng/ml to 50ng/ml mouse Resistin (10 µl sample size). Any result greater than 50 ng/ml in a 10 µl sample should be diluted using matrix solution, and the assay repeated until the results fall within range. Adipocyte extracts or cell culture media samples greater than 50 ng/ml in a 10µl sample should be diluted in Assay Buffer.

STANDARD CURVE



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Mouse Resistin that can be detected by this assay is 0.78 ng/ml when using a 10 µl sample size.

B. Specificity

The antibody pair used in this assay is specific to mouse resistin and does not significantly cross-react with human or rat resistin and other cytokine or hormone molecules tested, as shown in the following table:

Analyte	Max. Conc.	Cross Reactivity
Mouse Resistin		100%
Human Resistin	10ng/ml	n.d.
Rat Resistin	100ng/ml	n.d.
Mouse Adiponectin	100ng/ml	n.d.
Mouse Cytokines:		
1. IL-1ß	10ng/ml	n.d.
2. IL-2	10ng/ml	n.d.
3. IL-4	10ng/ml	n.d.
4. IL-5	10ng/ml	n.d.
5. IL-6	10ng/ml	n.d.
6. IL-9	10ng/ml	n.d.
7. IL-10	10ng/ml	n.d.
8. IL-12	10ng/ml	n.d.
9. IL-13	10ng/ml	n.d.
11.IFN-γ	10ng/ml	n.d.
12.TNFα	10ng/ml	n.d.
13.GMCSF	10ng/ml	n.d.
14.MIP-1α	10ng/ml	n.d.
15.MCP-1	10ng/ml	n.d.
16.KC	10ng/ml	n.d.
17.RANTES	10ng/ml	n.d.
Mouse Endocrine Hormones		
1. Insulin	10pM	n.d.
2. Amylin	10pM	n.d.
3. Leptin	10pM	n.d.
4. Glucagon	10pM	n.d.
5. GLP-1	10pM	n.d.
Human Endocrine Hormones		
1. Insulin	10pM	n.d.
2. Amylin	10pM	n.d.
3. Leptin	10pM	n.d.
4. Glucagon	10pM	n.d.
5. GLP-1	10pM	n.d.
6. C-Peptide	10pM	n.d.

n.d. = not detectable

C. Precision

Within and Between Assay Variation

Sample No.	Mean Resistin Levels (ng/ml)	Within% CV	Between% CV
1	1.83	2.96	10.3
2	5.36	3.21	6.97
3	5.73	5.41	4.99
4	12.11	4.57	7.5

The assay variations of the Resistin ELISA kits were studied on four mouse serum samples with varying concentrations of endogenous Resistin. The mean within assay variation was calculated from results of six duplicate determinations in each assay of the indicated samples. The mean between assay variations of each sample was calculated from results of four separate assays with duplicate samples in each assay.

D. Recovery

Spike & Recovery of Mouse Resistin in Serum

Sample No.	Resistin Added (ng/ml)	Expected (ng/ml)	Observed (ng/ml)	% of Recovery
1	0	2.02	2.02	100
	1.56	3.58	3.65	102
	6.25	8.27	7.84	95
	12.5	14.52	13.27	91
2	0	5.71	5.71	100
	1.56	7.27	7.37	101
	6.25	11.96	10.91	91
	12.5	18.21	16.66	91
3	0	11.91	11.91	100
	1.56	13.47	12.68	94
	6.25	18.16	16.38	90
	12.5	24.41	23.14	95

Varying amounts of mouse Resistin were added to three mouse serum samples and the Resistin content was determined in three separate assays. The % of recovery = observed Resistin concentrations/expected Resistin

concentrations x 100%.

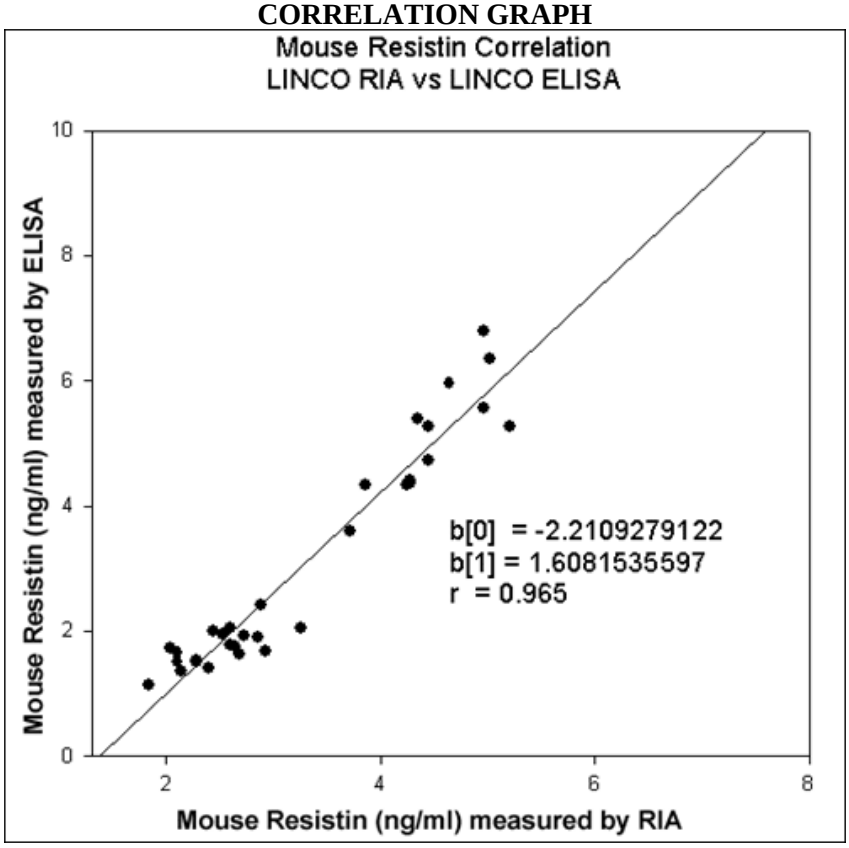
A. Linearity

Effect of Serum Dilution

Sample No.	Volume Sampled (µl)	Expected (ng/ml)	Observed (ng/ml)	% Of Expected
1	10	6.4	6.4	100
	5	3.2	3.2	100
	2.5	1.6	1.66	104
	1.67	1.07	1.21	113
2	10	7.62	7.62	100
	5	3.81	3.8	97
	2.5	1.91	1.85	100
	1.67	1.27	1.24	100
3	10	11.51	11.51	100
	5	5.76	5.31	92
	2.5	2.88	3.08	107
	1.67	1.92	1.83	95

Three mouse serum samples with the indicated sample volumes were assayed in three separate experiments. Required amounts of matrix were added to compensate for lost volumes below 10 µl. The resulting dilution factors of 1.0, 2.0, 4.0, and 6.0 representing 10 µl, 5 µl, 2.5 µl, and 1.67µl sample volumes assayed, respectively, were applied in the calculation of observed Resistin concentrations. % expected = observed/expected x 100%.

CORRELATION GRAPH



Serum and plasma samples obtained from 32 mice were assayed for Resistin content using both Mouse Resistin RIA Kit and Mouse Resistin ELISA Kit. Correlation of the two kits is derived by linear regression analysis of paired results from each sample.

QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert.

TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.

2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.

3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.

4. Avoid cross contamination of any reagents or samples to be used in the assay.

5. Make sure all reagents and samples are added to the bottom of each well.

6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete

mixing or cross well contamination due to inappropriate mixing.

7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.

8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.

9. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with Wash Buffer or 3) overexposure to light after substrate has been added.

REPLACEMENT REAGENTS

Reagents
Mouse Resistin ELISA Plate
10X HRP Wash Buffer Concentrate (50 ml)
Mouse Resistin Standards
Mouse Resistin Quality Controls 1 and 2
Assay Buffer
Matrix Solution
Mouse Resistin Detection Antibody
Enzyme Solution
Substrate
Stop Solution

Vers. 11/28/05
~sd