Mouse Resistin immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Mouse Resistin in cell culture supernates, serum, and EDTA plasma. It contains recombinant Mouse Resistin and antibodies raised against this protein. It has been shown to accurately quantitie recombinant Mouse Resistin. Results obtained with naturally occurring Resistin samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the Immunoassay kit can be used to determine relative mass values for natural Mouse Resistin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Mouse Resistin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any resistin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for resistin is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of resistin bound in the initial step. The color development is stopped and the intensity of the color is measured.

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

All reagents should be considered as potentially hazardous. The stop solution contains diluted Hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

DESCRIPTION	CODE	QUANTITY
RESISTIN Microplate – 96 well microplate precoated with monoclonal anti-Mouse Resistin, one plate	100-03-01	1 plate
RESISTIN Standard – 1 ng/vial of recombinant Mouse Resistin in a buffered protein base with preservatives; lyophilized.	100-03-02	1 vial
RESISTIN Antibody Concentrate– 105µl / vial, 100-fold concentrated of polyclonal Antibody against Mouse Resistin with preservatives; lyophilized.	100-03-03	1 vial
Positive Control – one vial of recombinant Mouse Resistin , lyophilized (optional)	100-03-04	1 vial
Streptavidin-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer- 60 mL/vial of buffered protein based solution with preservatives	DB01	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with	WB01	1 vial

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Antibody Concentrated should be stored at -20 or -70 °C. Do not use past kit expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Positive Control and Antibody SHOULD BE STORED at -20 °C or – 70°C for up to one months. Streptavidin - HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 4 months at 2 - 8° C.

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Serum and EDTA plasma samples require a 50-100 fold dilution. A suggested 50-fold dilution is 5 µL sample + 245 µL Dilution Buffer. A suggested 100-fold dilution is 5 µL sample + 495 µL Dilution Buffer. Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

RESISTIN Standard - Refer to vial label for reconstitution volume. Reconstitute the **RESISTIN** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer DB01 serves as the zero standard (0 pg/mL).

STANDARD TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1000 pg/ml
# 1	250µl of stock	250µl	500 pg/ml
# 2	250µl of 1	250µl	250 pg/ml
# 3	250µl of 2	250µl	125 pg/ml
# 4	250µl of 3	250µl	62.5 pg/ml
# 5	250µl of 4	250µl	31.25 pg/ml
# 6	250µl of 5	250µl	15.6 pg/ml

RESISTIN Antibody- Reconstitute the **Antibody concentrated** with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Transfer it to 10.315 mL of Dilution Buffer to prepare 1 x Antibody solution.

250 µl	2 µl	2 µl	2 µl	2 µl	250 µl

1 mL

Standard	1	2	3	4	5	6	
Concen	1000	500	2	1	6	3	15.6	l

Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 12 ml of Dilution Buffer to prepare working solution. Note: 1 x working solution of Streptavidin HRP Conjugate should be used within a few days.

Positive Control- Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. Positive Control should be prepared and used immediately.

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Leave well B2 and B3 as Blank. Add 100 µl per well of Dilution Buffer.
4. Add 100 µl per well of standard solution from #6 to #S (reverse order of serial dilution) to the appropriate wells (C2 to F3, F4 to D5). Add 100 µl per well of Positive control into well C4 and C5. Add 100 µl per well of samples into appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm). Note: Standard, Blank and PC should be assayed in duplicate.
5. Aspirate wells and wash 5 times with 300 µl of 1 x Assay Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1 x Antibody solution. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate** working solution. Cover or seal the plate and incubate at room temperature for 45 minutes on microplate shaker.
11. Repeat the aspiration/wash as in step 5.
12. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 minutes at room temperature. **Protect from light**.
13. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Average the duplicate readings for each standard, QC , and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a log-log curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant Mouse Resistin .

The minimum detectable dose (MDD) of Mouse Resistin Was 7.8 pg/mL.

To assess the linearity of the assay, pooled research mouse EDTA plasma samples were diluted with Dilution Buffer DB01 and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
50X	820.692	41.03	100
100X	367.418	36.74	89.5

These standard curves * are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	OD450 READING

This assay recognizes both natural and recombinant Mouse Resistin. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY
Mouse Resistin	100
Mouse RELM-alpha	0
Human Resistin	0
Human RELM-beta	0
Mouse Adiponectin	0
Mouse Vaspin	0
Mouse FGF-21	0