DRG® Resistin (Mouse) (EIA-4554)

Revised 4 Jan. 2007 **For Veterinary Use Only**

1 INTENDED USE

The Mouse Resistin ELISA is a biotin labelled antibody based sandwich enzyme immunoassay for the quantitative measurement of mouse resistin in serum, plasma (EDTA, citrate) and tissue culture medium. It is intended for in vitro research use only.

Features

- The total assay time is less than four hours.
- Quality control is mouse serum based.
- Components of the kit are ready-to-use (with the exception of Dilution Buffer and Wash Solution).

2 SUMMARY

Resistin, a product of the RSTN gene, is a peptide hormone belonging to the class of cysteine-rich secreted proteins (monomeric peptide contains 11 cysteine residues) referred to as the RELM family, and is also described as ADSF (Adipose Tissue-Specific Secretory Factor) or FIZZ3 (Found in Inflammatory Zone 3). Mouse resistin is expressed as a 114 amino acid prepeptide; its hydrofobic N-terminal 20 amino acid signal peptide is cleaved before its secretion. Mouse resistin circulates in blood as a homodimeric protein consisting of two 94 amino acid polypeptides, which are disulfide-linked via Cys26.

Resistin may be an important link between obesity and insulin resistance. Mouse resistin, specifically produced and secreted by adipocyte, acts on skeletal muscle myocytes, hepatocytes and adipocytes themselves so that it reduces their sensitivity to insulin. Steppan et al. have suggested that resistin suppressed the ability of insulin to stimulate glucose uptake. They have also suggested that resistin was present at elevated levels in blood of obese mice, and was down regulated by fasting and by antidiabetic drugs. Way et al., on the other hand, have found that resistin expression is severely suppressed in obesity and is stimulated by several antidiabetic drugs.

Other studies have shown that mouse resistin increases during the differentiation of adipocytes, but it also seems to inhibit adipogenesis. In contrast, the human adipogenic differentiation is likely to be associated with a down regulation of resistin gene expression.

3 PRINCIPLE OF THE TEST

In the DRG®'s Mouse Resistin ELISA, calibrators or samples are incubated with a goat polyclonal anti-mouse resistin antibody coated in microtiter wells. The wells are washed and biotin-labelled polyclonal anti-mouse resistin antibody is added and incubated with captured resistin. After a thorough wash, streptavidin-horseradish peroxidase conjugate is added. After the following incubation and washing steps, the conjugate bound is allowed to react with the substrate H_2O_2-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of resistin. A standard curve is constructed by plotting absorbance values versus resistin concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

4 PRECAUTIONS

For in vitro and research use only.

- This kit contains components of animal origin.
- Wear gloves and laboratory coats when handling immunodiagnostic materials and samples.
- Avoid contact with the acid Stop Solution and Substrate Solution, which contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- Reagents with different lot numbers should not be mixed.
- Reagents should not be used after the expiry specified on the kit label.

5 REAGENTS SUPPLIED

1. **Microtiter Strips** (96 wells coated with capture Anti-Resistin Antibody, vacuum sealed)
2. Biotin Labelled Anti-Resistin **Antibody**, ready to use, 13 ml
3. Streptavidin-HRP **Conjugate**, ready to use, 13 ml
4. **Substrate (TMB) Solution**, 13 ml
5. **Stop Solution** (0.2 M H_2SO_4), 13 ml
6. **Dilution Buffer** Concentrate (5x), 22 ml
7. Mouse Resistin **Standards** (
   1, 2, 5, 10, 20, 50 and 100 ng/ml); 0.05 ml each concentration
8. Quality **Control**, lyophilizate
   (refer to the Certificate of Analysis for actual Quality Control value)
9. **Wash Solution** Concentrate (10x), 100 ml

6 MATERIALS REQUIRED BUT NOT SUPPLIED

- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 µL and disposable tips
- Multichannel pipette 100 µL and disposable tips
- Microplate reader with 450 ± 10 nm filter
- Software package facilitating data generation and analysis
- Orbital microplate shaker capable of approximately 300 rpm (optional)
- Microtitration plate washer (optional) [Manual washing is possible but not preferable.]
- Absorbent material for blotting the microtiter plate
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water

7 PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to the assay.

Assay reagents are supplied ready-to-use, with the exception of Wash Solution Concentrate.

Dilution Buffer:
Dilute 22 ml of Dilution Buffer Concentrate with 88 ml of deionized (distilled) water.
The working Wash Solution is stable for 1 month at 2-8 °C.

Wash Solution:
Dilute 100 ml of Wash Solution Concentrate with 900 ml of deionized (distilled) water.
The working Wash Solution is stable for 1 month at 2-8 °C.

Reconstitution of Quality Control:
Add 50 µL of deionized (distilled) water to the vial containing lyophilized Quality Control, let it dissolve for at least 15 minutes and mix thoroughly.
The reconstituted control serum has to be used immediately or to be stored frozen.

8 PREPARATION OF STANDARDS AND SAMPLES

Standards:
Dilute Standards 1:100 with Dilution Buffer prior to use (preferably 10 µL standard + 990 µL Dilution Buffer).

Quality Controls:
Dilute the reconstituted Quality Control 1:100 with Dilution Buffer (preferably 10 µL control + 990 µL Dilution Buffer).

Samples:
Dilute serum or plasma samples 1:100 with Dilution Buffer (preferably 10 µL sample + 990 µL Dilution Buffer for duplicates).

9 STORAGE AND STABILITY OF SAMPLES

Serum samples should be stored frozen (preferably at -80 °C, then the stability is at least 1 year).
Repeated thawing-freezing cycles should be avoided.

Undiluted samples are stable at least 1 week at 2-8 °C or 1 day at RT.

Diluted samples have to be stored frozen.

10 ASSAY PROCEDURE

1. Pipet 100 µL of diluted Standards, Quality Controls and samples, preferably in duplicates, into the appropriate wells.
2. Incubate the plate for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker at RT.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well).
4. Add 100 µL of Biotin-Labelled Antibody solution.
5. Incubate the plate for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker at RT.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well).
7. Add 100 µL of Streptavidin-HRP Conjugate solution.

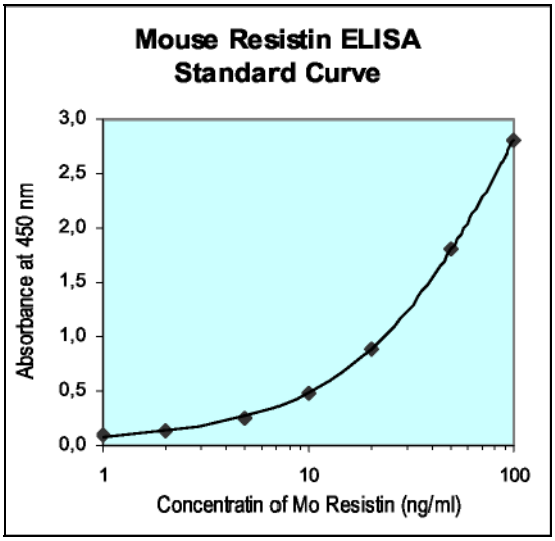
8. Incubate the plate for 30 min, shaking at ca. 300 rpm on an orbital microplate shaker at RT.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well).
10. Add 100 µL of Substrate Solution. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
11. Incubate the plate for 10-15 min at RT.
12. Stop the colour development by adding 100 µL of Stop Solution.
13. Determine the absorbance by reading the plate at 450 nm. (optionally, to measure in dual wavelength mode 620-650 nm filter can be used to measure the reference absorbance. The absorbance should be read within 5 minutes following step 12).

Note:
If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. a new standard curve, constructed using the values measured at 405 nm, is used to determine Resistin concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

11 CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of standards versus log of the known concentration (X) of standards, using the four-parameter function. Results are reported as concentration of mouse resistin (ng/ml) in samples.

Alternatively, the logit log function can be used to linearize the calibration curve (i.e. logit of absorbance (Y) is plotted versus log of the known concentration (X) of standards).



12 LIMITS OF ASSAY

Results exceeding 100 ng/ml should be repeated with more diluted samples (e.g. 1:200). Dilution factors (e.g. 2) need to be taken into consideration in calculating the Resistin concentration.

13 STORAGE, EXPIRATION

Store the kit at 2-8°C. Under these conditions, assay components are stable till the expiry date is over. (See the expiry date indicated on the kit label).

14 PERFORMANCE CHARACTERISTICS

Typical analytical data of DRG Human Resistin ELISA are presented in this chapter.

For actual Standard curve and Quality Controls values see the Certificate of Analysis.

14.1 Sensitivity

The limit of detection (defined as mouse Resistin concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is defined as follows:

Analytical Limit of Detection is calculated from the real Resistin values in wells and is 5 pg/ml.

Assay Sensitivity takes the dilution of samples into consideration and is calculated according to the formula:

Assay Sensitivity = Analytical Limit of Detection x sample dilution
= 5 pg/ml x 100 = 0.5 ng/ml

*DILUTION BUFFER IS PIPETTED INTO BLANK WELLS.

14.2 Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	20.82	0.99	4.8
2	48.44	2.09	4.3

Inter-assay (Run-to-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	51.80	2.90	5.6
2	82.12	4.29	5.3

14.3 Spiking Recovery

Serum samples were spiked with different amounts of recombinant mouse Resistin and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	6.22	-	-
	10.76	11.22	96
	16.00	16.22	99
	24.06	26.22	92
2	24.90	-	-
	31.56	29.90	106
	36.82	34.90	106
	46.00	44.90	102

14.4 Linearity

Serum samples (100 times diluted) were further serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	47.03	-	-
	1:2	24.00	23.52	102
	1:4	11.98	11.76	102
	1:8	6.44	5.88	110
2	-	78.11	-	-
	1:2	39.45	39.06	101
	1:4	19.96	19.53	102
	1:8	9.94	9.76	102

14.5 Normal Values

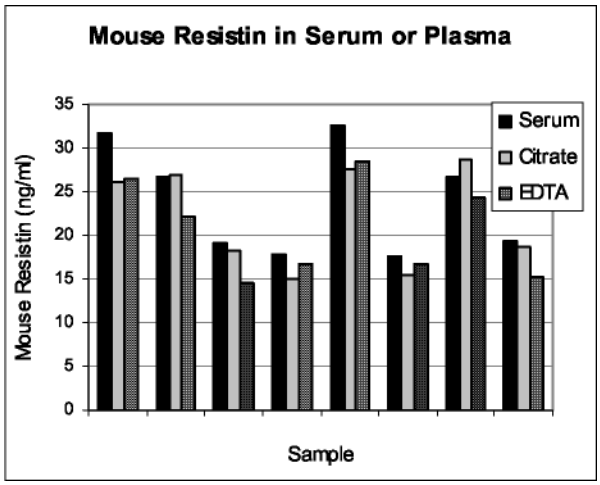
The following values were obtained when 61 sera from healthy BALB/c mice were assayed:

Mouse (BALB/c) (n=61)	**Mouse Resistin** (ng/ml)
Mean	27.1
Median	26.7
Normal Range (Mean± 2*SD)	7.5 – 46.6

14.6 Serum/ Plasma Samples

Citrate and EDTA plasmas were compared to respective serum samples obtained from healthy BALB/c mice (n = 8) in the same time.

Sample (n = 8)	Mean Mouse Resistin (ng/ml)	Plasma/Serum ± SD
Serum	23.96	-
Citrate Plasma	22.10	92 ± 9 %
EDTA Plasma	20.57	86 ± 7 %



14.7 Specificity

- Sera of several mammalian species were measured in the assay giving the following signal equivalent to Mouse Resistin: Human (0.30 ng/ml), Rat (2.43 ng/ml), Rabbit (0.28 ng/ml), Cow (0.19 ng/ml) and Horse (2.04 ng/ml).
- The assay recognizes natural and recombinant Mouse Resistin. No cross-reactivity has been observed for Mouse cytokines: RELM-α, RELM-β, Leptin, Leptin Receptor and Adiponectin at 100 ng/ml.

15 TROUBLESHOOTING AND FAQS

A. Weak signal in all wells

- Omitting a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to reach room temperature

B. High signal and background in all wells Possible explanations:

• Improper or inadequate washing
• Overdeveloping; incubation time should be reduced before addition of Stop Solution

C. High coefficient of variation (CV) Possible explanation:

• Improper or inadequate washing

16 REFERENCES

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SYMBOLS USED WITH DRG® ELISA´S

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	Research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
Σ		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..