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## MOUSE RESISTIN ENZYME IMMUNOASSAY KIT

catalogue # A05178

96 wells

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*For research laboratory use only.  
Not for diagnostic use.*



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## MOUSE RESISTIN EIA KIT

96 wells  
Storage: 2-8°C  
Expiry date: stated on the package

This kit contains:

- ☞ A covered 96 well plate, pre-coated with a rabbit polyclonal anti-mouse Resistin antibody, ready to use
- ☞ One vial of biotin labelled anti-mouse Resistin antibody, ready to use
- ☞ One vial of Streptavidin tracer, ready to use
- ☞ Seven vials of mouse Resistin standards (1, 2, 5, 10, 20, 50, 100 ng/mL), ready to use
- ☞ One vial of Substrate (TMB) solution, ready to use
- ☞ One vial of Stop solution (0.2 M H<sub>2</sub>SO<sub>4</sub>), ready to use
- ☞ One vial of concentrated EIA Buffer (5x), liquid
- ☞ One vial of mouse Resistin Quality Control, lyophilised
- ☞ One vial of concentrated Wash buffer (10x), liquid
- ☞ One instruction booklet
- ☞ One template sheet
- ☞ One well cover sheet

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 40 samples in duplicate.

### PRECAUTIONS FOR USE

**Users are recommended to read all instructions for use before starting work.**

Each time a new pipet tip is used, aspirate a sample of reagent and dispense into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.

Not for diagnostic use.

Do not pipet liquids by mouth.

Do not use kit components beyond the expiration date.

Do not mix different lot numbers.

Do not eat, drink, or smoke in area in which kit reagents are handled.

Avoid splashing.

Wear gloves and laboratory coats are recommended when handling immunodiagnostics materials and samples of human origin.

Stop solution and Substrate solution are potential harmful solution. To avoid any contact, wear eye, hand, face and clothing protection when handling these reagents.

### PRINCIPLE OF THE ASSAY

Resistin is a protein hormone with important effects in metabolism and regulating body weight. 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 hydrophobic 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. Stepan 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.

This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a rabbit polyclonal antibody specific of mouse Resistin. This antibody will bind any mouse Resistin introduced in the wells (sample or standard).

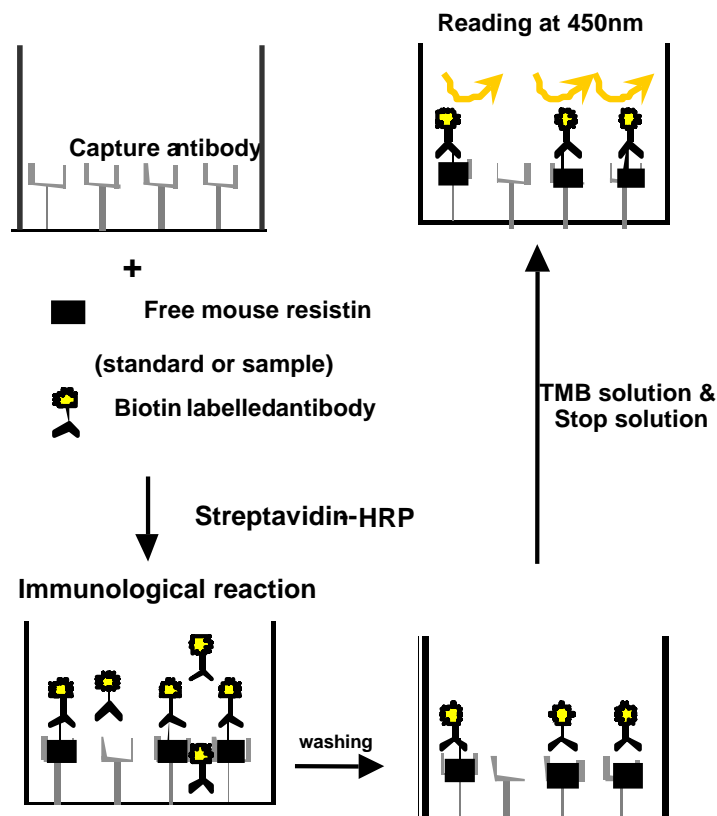
After one-hour incubation and a washing, biotin-labelled polyclonal anti-mouse Resistin antibody is added and incubated with captured Resistin during one hour.

After a thorough wash, streptavidin-horseradish peroxidase tracer is added and incubated for one hour. This allows the two antibodies to form a sandwich by binding on different parts of the Resistin molecule.

The sandwich complex is immobilised on the plate so the excess reagents may be washed away. The concentration of the Resistin is then determined by measuring the enzymatic activity of the HRP using the hydrogen peroxide/TMB solution. The reaction is stopped by addition of sulfuric acid solution. The HRP tracer acts on TMB Reagent to form a yellow compound.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of the resistin present in the well during the immunological incubation.

The principle of the assay is summarised below:





## MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

### **FOR THE ASSAY**

- ☞ Precision micropipettes (10 to 1000 µL)
- ☞ Spectrophotometer plate reader (450 nm +/- 10 nm filter)
- ☞ Microtitration washer (or washbottles)
- ☞ Microplate shaker
- ☞ Multichannel pipette 100 µL and disposable tips
- ☞ Distilled or deionised water
- ☞ Polypropylene tubes

## SAMPLE PREPARATION

This assay may be used to measure resistin in mouse samples such as plasma (EDTA, citrate), serum and tissue culture supernatant.

### **GENERAL PRECAUTIONS**

- ☞ All samples must be free of organic solvents prior to assay.
- ☞ Samples should be assayed immediately after collection or should be stored at -20°C, preferably -80°C.

### **SAMPLE PREPARATION**

Dilute samples 100 times in EIA buffer (i.e. 10 µL sample + 990 µL EIA buffer).  
Do not store diluted samples.

## REAGENT PREPARATION

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready to use, except the Quality Control, EIA buffer and Wash buffer.

- ☞ EIA buffer  
Reconstitute one vial of concentrated EIA buffer (22 mL, 5x) to 110 mL with distilled or deionised water.
- ☞ Quality Control  
Reconstitute the vial with 50 µL of distilled or deionised water. Mix thoroughly by gentle inversion. Let stand for 15 minutes.  
The reconstituted QC has to be used immediately or to be frozen.  
Dilute mouse Resistin Quality Control 100 times in EIA buffer (i.e. 10 µL QC + 990 µL EIA buffer).
- ☞ Mouse Resistin Standards  
Dilute mouse Resistin standards 100 times in EIA buffer (i.e. 10 µL standard + 990 µL EIA buffer).
- ☞ Wash buffer  
Reconstitute one vial of concentrated Wash buffer (100 mL, 10x) to 1000 mL with distilled or deionised water.  
Stability at 4°C: 1 month
- ☞ Hydrogen peroxide/TMB solution  
Substrate solution should remain colourless until added to the plate. Keep substrate solution protected from the light.

## ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

### **DISTRIBUTION OF REAGENTS AND SAMPLES**

A plate set-up is suggested on the following page. The content of each well may be recorded on the sheet provided with the kit.

### **PIPETTING THE REAGENTS**

All samples and reagents must reach room temperature prior performing the assay. Use different tips to pipet the buffer, standard, sample, tracer antiserum and other reagents.

#### ↪ Mouse Resistin standard:

Dispense 100  $\mu$ L of each diluted standard (1 to 100 ng/mL) in duplicate to appropriate wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.

#### ↪ Quality Control and samples:

Dispense 100  $\mu$ L of diluted Quality Control and diluted samples in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

#### ↪ EIA buffer:

Dispense in duplicate 100  $\mu$ L of EIA buffer in Blank (B) wells.

	12	*	*	*	*	*	*	*	*
	11	*	*	*	*	*	*	*	*
	10	*	*	*	*	*	*	*	*
	9	*	*	*	*	*	*	*	*
	8	*	*	*	*	*	*	*	*
	7	*	*	*	*	*	*	*	*
	6	*	*	*	*	*	*	*	*
	5	*	*	*	*	*	*	*	*
	4	*	*	*	*	*	*	*	*
	3	*	*	*	*	*	*	*	*
	2	S4	S4	S5	S5	S6	S6	S7	S7
1		B	B	S1	S1	S2	S2	S3	S3
		<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>

B: Blank

S1-S7: Standards 1-7

\*: Samples or Quality Controls

### **INCUBATING THE PLATE**

↪ Cover the plate with the cover sheet and incubate the plate for 1 hour, shaking at 300 rpm on an orbital microplate shaker.

↪ Rinse the wells 3 times with the Wash buffer (350  $\mu$ L per well).

↪ Biotin-labelled anti-mouse Resistin antibody:  
Dispense 100  $\mu$ L to each well.

- ↪ Cover the plate with the cover sheet and incubate the plate for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- ↪ Rinse the wells 3 times with the wash buffer (350 µL per well).
- ↪ Streptavidin-HRP tracer:  
Dispense 100 µL to each well.
- ↪ Cover the plate with the cover sheet and incubate the plate for 30 minutes, shaking at 300 rpm on an orbital microplate shaker.
- ↪ Rinse the wells 3 times with the wash buffer (350 µL per well).

**DEVELOPING AND READING THE PLATE**

- ↪ Dispense 100 µL of Substrate solution to the 96 wells. Incubate in the dark during 10 minutes at room temperature (20-30 °C). Avoid exposure to direct sunlight. It is recommended to cover the plate with aluminium foil.
- ↪ Stop the colour development by adding 100 µL of Stop solution.
- ↪ Read the absorbance at 450 nm within 5 minutes following stop solution addition.

*Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the lowest standard (the highest absorbance of the calibration curve), 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.***

Enzyme Immunoassay Protocol (Volumes are in µL)			
	Blank	Standard	Sample
EIA Buffer	100		
Standard	-	100	-
Sample	-	-	100
Incubate the plate at room temperature for 1 hour Wash the plate 3 times			
Biotin Labelled Resistin antibody	-	100	100
Incubate the plate at room temperature for 1 hour Wash the plate 3 times			
Streptavidin-HRP	100	100	100
Incubate the plate at room temperature for 30 minutes Wash the plate 3 times			
TMB solution	100	100	100
Incubate the plate in the dark at room temperature during 10 minutes			
Stop solution	100	100	100
Read the plate at 450 nm			

**DATA ANALYSIS**

Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of TMB solution) from the absorbance readings of the rest of the plate. If not, do it now.

- ↪ Using a logit-log graph paper, plot the absorbance for each standard (y axis) versus concentration (x axis) of standards. Draw a best-fit line through the points.

- ↪ To determine the concentration of your samples, find the absorbance value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample.
- ↪ Most plate readers are supplied with curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

## TYPICAL DATA

### EXAMPLE DATA

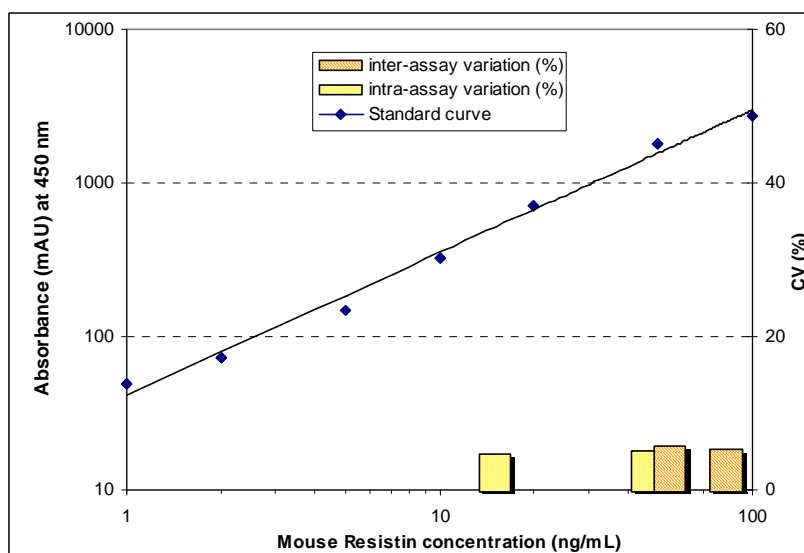
The following data are for demonstration purposes only. Your data may be different but still be correct. These data were obtained using all reagents as supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Mouse Resistin	mAU
Blank	33
Standard 100 ng/mL	2 758
Standard 50 ng/mL	1 781
Standard 20 ng/mL	704
Standard 10 ng/mL	321
Standard 5 ng/mL	147
Standard 2 ng/mL	72
Standard 1 ng/mL	49
QC	834

### ACCEPTABLE RANGE

- ↪ QC samples: see label on the vials.

## MOUSE RESISTIN STANDARD CURVE



## ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of mouse Resistin has been validated for its use in serum, plasma (EDTA, citrate) and culture supernatant.

### ☞ Cross-reactivity:

- Mouse Leptin	<0.1%
- Mouse Leptin receptor	<0.1%
- Mouse Adiponectin	<0.1%
- Human serum equivalent to	0.30 ng/mL
- Rat serum equivalent to	2.43 ng/mL
- Rabbit serum equivalent to	0.28 ng/mL
- Bovine serum equivalent to	0.19 ng/mL
- Horse serum equivalent to	2.04 ng/mL

### ☞ Sensitivity:

The limit of detection (defined as such a concentration of mouse Resistin giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank:  $A_{\text{blank}} + 3 \cdot SD_{\text{blank}}$ ) is better than 0.5 ng/mL of sample. The EIA buffer was pipetted into blank wells, and the microtiter plate is blanked on air.

### ☞ Precision:

#### • Intra-assay (n=8)

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

#### • Intra-assay (n=8)

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

### ☞ Recovery test:

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

### ☞ Dilution test:

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



☞ Normal values:

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

Mouse (BALB/c) (n=61)	Mouse Resistin (ng/mL)
Mean	27.1
Median	26.7
Normal range ( Mean $\pm$ 2 SD)	7.5 – 46.6

☞ Serum/ Plasma Samples:

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

Sample (n = 8)	Mean Resistin (ng/mL)	Plasma /Serum (%) +SD (%)
Serum	23.96	-
Citrate Plasma	22.10	92 $\pm$ 9
EDTA Plasma	20.57	86 $\pm$ 7

### ASSAY TROUBLE SHOOTING

☞ Absorbance values too low:

- One reagent has not been dispensed
- Incorrect preparation or reagent storage
- Assay performed before reagents reach room temperature

☞ High signal and background in all wells:

- Inefficient washing
- Overdeveloping; incubation time should be reduced before adding Stop Solution

☞ High dispersion of duplicates:

- Poor pipetting technique or irregular plate washing.

These are a few examples of problems that may occur. If you need further assistance, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (sales@spibio.com), and be sure to indicate the lot number of the kit (see outside of the box).

SPI-BIO offers a training workshop in EIA practice & theory. This workshop is given twice a year. For further information, please contact our Customer Relation Representative (33 (0)1 39 30 62 60).

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