

RAT RESISTIN ENZYME IMMUNOASSAY KIT

catalogue # A05179

96 wells

TABLE OF CONTENTS

Presentation	1
Precautions for use	1
Principle of the assay	1
Materials and equipment required	3
Sample preparation	3
General precautions	3
Sample preparation	3
Reagent preparation	3
Assay procedure	4
Distribution of reagents and samples	4
Pipetting the reagents	4
Incubating the plate	5
Developing and reading the plate	5
Data analysis	6
Typical data	6
Example data	6
Acceptable range	6
Rat Resistin standard curve	6
Assay validation and characteristics	7
Assay trouble shooting	8
Bibliography	8

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



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RAT 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-rat Resistin antibody, ready to use
- ◆ One vial of biotin labelled anti-rat Resistin antibody, ready to use
- ◆ One vial of Streptavidin tracer, ready to use
- ◆ One vial of rat Resistin standard, lyophilised, 20 ng
- ◆ One vial of Substrate (TMB) solution, ready to use
- ◆ One vial of Stop solution (0.2 M H₂SO₄), ready to use
- ◆ One vial of EIA Buffer,
- ◆ Two vials of rat Quality Controls, low and high, 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 41 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 which is termed the RELM family, and is also described as ADSF (Adipose Tissue-Specific Secretory Factor) and FIZZ3 (Found in Inflammatory Zone). Rat resistin contains 114 amino acids as a prepeptide, and its hydrophobic signal peptide is cleaved before its secretion. Resistin circulates in rat blood as a dimeric protein consisting of two 96 amino acid polypeptides, which are disulfide-linked.

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 suppresses the ability of insulin to stimulate glucose uptake. They have also suggested that resistin is

present at elevated levels in blood of obese mice, and is down regulated by fasting and antidiabetic drugs (TZDs). On the other hand, several studies demonstrated reduced resistin expression in adipose tissue of obese mice and increased levels in leptin deficient *ob/ob* mice and Zucker diabetic fatty rats in response to TZDs.

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 rat Resistin. This antibody will bind any rat Resistin introduced in the wells (sample or standard).

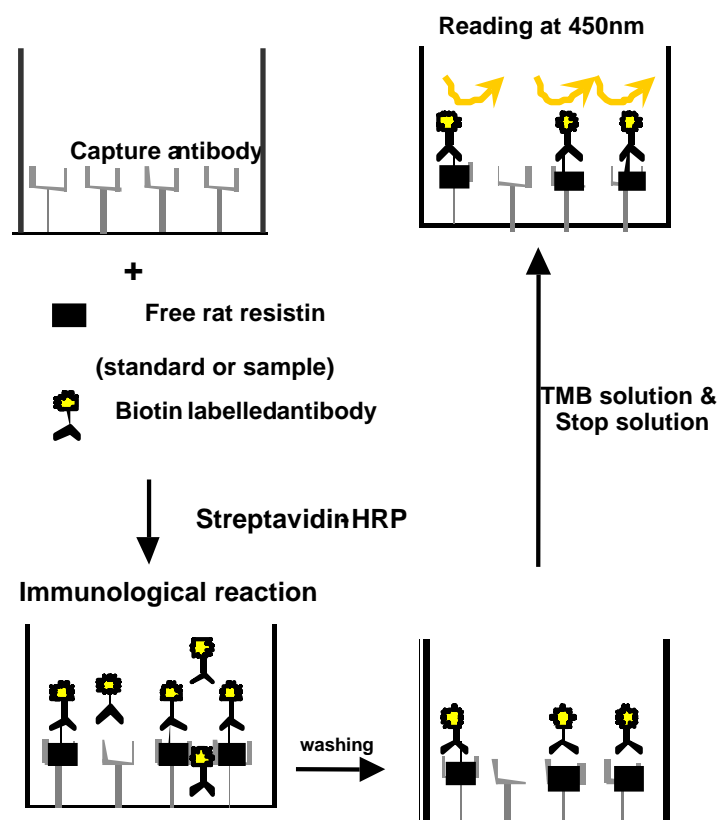
After one-hour incubation and a washing, biotin-labelled polyclonal anti-rat Resistin antibody is added and incubated with captured Resistin during one hour.

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 rat samples such as serum, plasma 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 20 times in EIA buffer (i.e. 20 µL sample + 380 µL EIA buffer).
Do not store the 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 Standard, Quality Control, Wash buffer.

- ◆ Quality Controls
Reconstitute the vials with 350 µL of EIA buffer. Mix thoroughly by gentle inversion. Let stand for 15 minutes.
The reconstituted QC has to be used immediately or to be frozen at -20°C.
- ◆ Rat Resistin Standards
Reconstitute Rat Resistin standard with X mL of EIA buffer as indicated on the vial label. The concentration of the rat resistin in the stock solution (S1) is 20 ng/mL.
Then prepare standards as follows:

Volume of Standards	Added volume of EIA buffer	Concentration of reconstituted standards
500 µL of S1	500 µL	S2 (10 ng/mL)
500 µL of S2	500 µL	S3 (5 ng/mL)
500 µL of S3	500 µL	S4 (2.5 ng/mL)
500 µL of S4	750 µL	S5 (1 ng/mL)
500 µL of S5	500 µL	S6 (0.5 ng/mL)
500 µL of S6	500 µL	S7 (0.25 ng/mL)

The reconstituted standards can be stored at -20°C until next use.

- ◆ 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.

- ↳ Rat Resistin standard:
Dispense 100 µL of each standard (S2 to S7: 0.25 to 10 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 µL of 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 µL of EIA buffer in Blank (B) wells.

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

B: Blank
S2-S7: Standards 2-7
*: 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 µL per well).
- ↪ Biotin-labelled anti-rat Resistin antibody:
Dispense 100 µL 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 conjugate:
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. Do not shake!
- ↪ 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 anti-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 5 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.
- ↳ As the standards and the Quality Controls are not diluted, while the samples are diluted 20 times, the values of samples calculated from the standard curve have to be multiplied by a dilution factor of 20 to obtain the true results.

TYPICAL DATA

EXAMPLE DATA

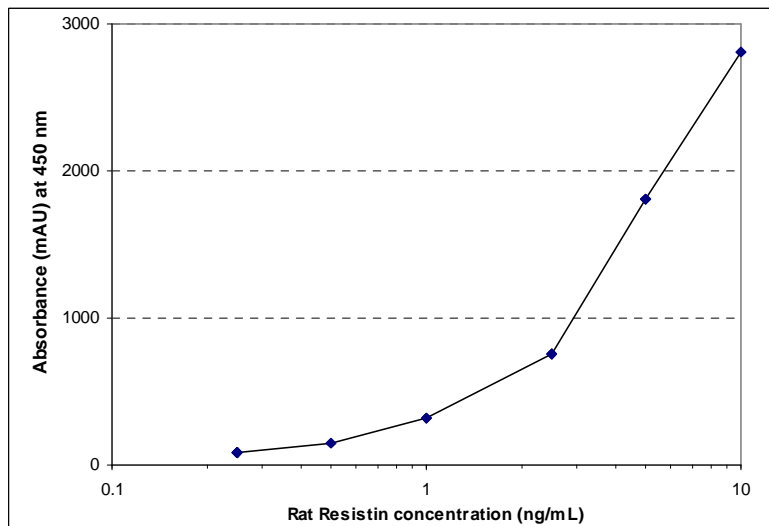
The following data are for demonstration purposes only. Your data may be different but still be correct. These data were obtained using all reagents as supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Rat Resistin	mAU
Blank	17
Standard 10 ng/mL	2 556
Standard 5 ng/mL	1 640
Standard 2.5 ng/mL	876
Standard 1 ng/mL	334
Standard 0.5 ng/mL	171
Standard 0.25 ng/mL	95
QC High	588
QC Low	209

ACCEPTABLE RANGE

◆ QC samples: see label on the vials.

RAT RESISTIN STANDARD CURVE



ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of Rat Resistin has been validated for its use in serum and tissue culture supernatant.

◆ Cross-reactivity:

- Human Resistin <0.1%
- Mouse Resistin <0.1%
- Rabbit Resistin <0.1%
- Horse Resistin <0.1%
- Goat Resistin <0.1%
- Pig Resistin <0.1%
- Sheep Resistin <0.1%
- Chicken Resistin <0.1%
- Hamster Resistin <0.1%
- Bovine Resistin <0.1%

◆ Sensitivity:

The limit of detection (defined as such a concentration of mouse/rat Resistin giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{\text{blank}} + 3*SD_{\text{blank}}$) is better than 0.05 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	14.07	0.73	5.2
2	21.12	1.03	4.9

◆ Recovery test:

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	14.45	-	-
	24.03	24.45	98.3
	30.83	34.45	89.5
	40.76	44.45	91.7
2	21.12	-	-
	28.00	31.12	90
	38.04	41.12	92.5
	64.50	71.12	90.7

◆ Dilution test:

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	28.25	-	-
	2x	15.89	14.12	112.5
	4x	8.03	7.06	113.7
2	-	26.32	-	-
	2x	15.13	13.16	114.9
	4x	7.21	6.58	109.5

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, Bertin Pharma 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 (bioreagent@bertinpharma.com), and be sure to indicate the lot number of the kit (see outside of the box). BertinPharma 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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