HUMAN RESISTIN

ENZYME IMMUNOASSAY KIT

catalogue # A05177

96 wells

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For research laboratory use only.
Not for diagnostic use.

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HUMAN RESISTIN EIA KIT

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

This kit contains:

- A covered 96 well plate, pre-coated with capture polyclonal anti-Resistin antibody, ready to use
- One vial of biotin labelled anti-Resistin antibody, ready to use
- One vial of Resistin standard, lyophilised, 50 ng
- Two vials of Quality Controls: low and high, lyophilised
- One vial of Streptavidin tracer, ready to use
- Two vials of EIA buffer, ready to use
- Two vials of Substrate solution (TMB), ready to use
- One vial of Stop solution (0.2 M H_2SO_4), ready to use
- One vial of concentrated Wash buffer (10x), liquid
- One instruction booklet
- One template sheet
- Three 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.

This kit contains components of human origin. These materials were found non-reactive for HbsAg, HCV antibody and for HIV 1/2 antibody and antigen. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents. 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 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).
Previous studies have shown that in mice, Resistin impairs glucose tolerance and insulin action and inhibits adipogenesis in murine 3T3-L1 cells. Therefore, Resistin has also been proposed as an adipocyte secreted factor that is thought to link obesity and type 2 diabetes. The role of Resistin in human obesity remains controversial even if some data show that thiazolinedione down regulate Resistin.

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 polyclonal antibody specific of human Resistin. This antibody will bind any Resistin introduced in the wells (sample or standard).

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

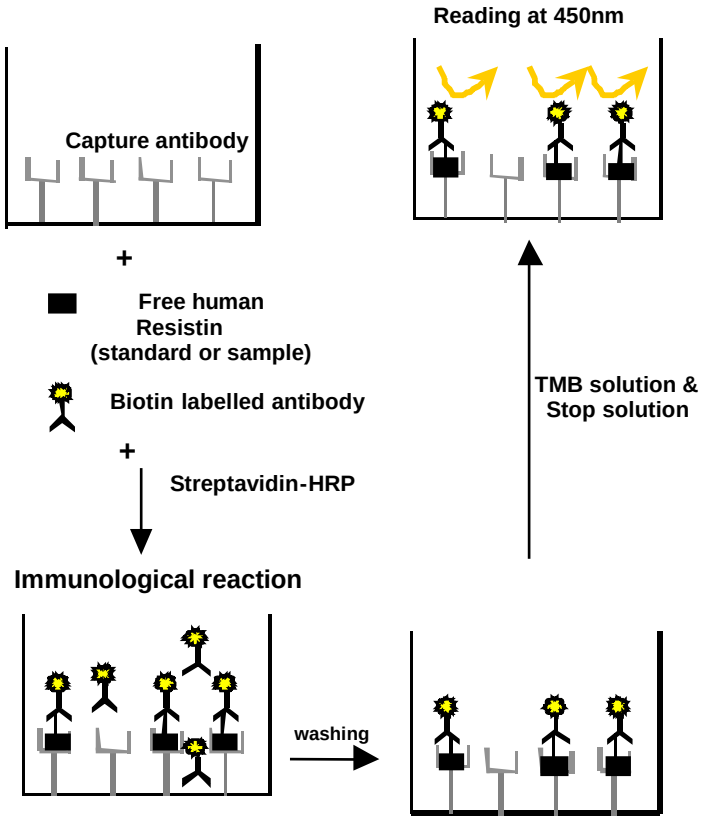
This allows the two antibodies to form a sandwich by binding on different parts of the human Resistin molecule.

After a thorough wash, streptavidin-horseradish peroxidase tracer is added.
The sandwich complex is immobilised on the plate so the excess reagents may be washed away.

The concentration of the human 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 human 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 human Resistin in human samples such as serum, plasma and 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.

SAMPLE PREPARATION

☞ No prior extraction procedure is necessary.

☞ To measure human Resistin, dilute serum or plasma samples 1/3 in EIA buffer (i.e. 100 µL sample + 200 µL EIA buffer).
Do not store the diluted samples.

Undiluted samples are stable at least one week at room temperature. No decline was observed in concentration of human Resistin in serum and plasma samples after repeated (3x) freezing/thawing cycles. However, repeated thawing-freezing cycles should be avoided.

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, the Quality Control, and concentrated Wash buffer.

☞ Human Resistin standard
Reconstitute Resistin standard with X mL of EIA buffer. The volume X is indicated on the vial of the corresponding standard. The concentration of the human Resistin in the stock solution (S1) is 50 ng/mL. Then prepare standards as follows:

Volume of Standards	Added volume of EIA buffer	Concentration of reconstituted Standards
500 µL of S1	750 µL	S2 (20 ng/mL)
500 µL of S2	500 µL	S3 (10 ng/mL)
500 µL of S3	500 µL	S4 (5 ng/mL)
500 µL of S4	750 µL	S5 (2 ng/mL)
500 µL of S5	500 µL	S6 (1 ng/mL)

Dilute reconstituted standards 1/3 in EIA buffer prior to use (i.e. 100 µL standard + 200 µL EIA buffer).
The reconstituted and undiluted standards could be frozen at -20°C until next use.
Do not store the diluted (1/3) standards.

☞ Quality Controls
Reconstitute Quality Control with X µL of EIA buffer. The volume X is indicated on the vial of the corresponding Quality Control. Mix the mixture and let it reconstitute thoroughly at room temperature for 30 minutes and mix again.
Dilute the reconstituted Quality Control 1/3 in EIA buffer prior to use (i.e. 100 µL Quality Control + 200 µL EIA buffer).
Reconstituted Quality Control is stable until the expiration date (see label on the box) when stored at -20°C.

☞ Wash buffer
Dilute 100 mL of concentrated Wash buffer to 1000 mL with distilled or deionised water.
The diluted Wash buffer is stable for one month when stored at 2-8°C.

☞ 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

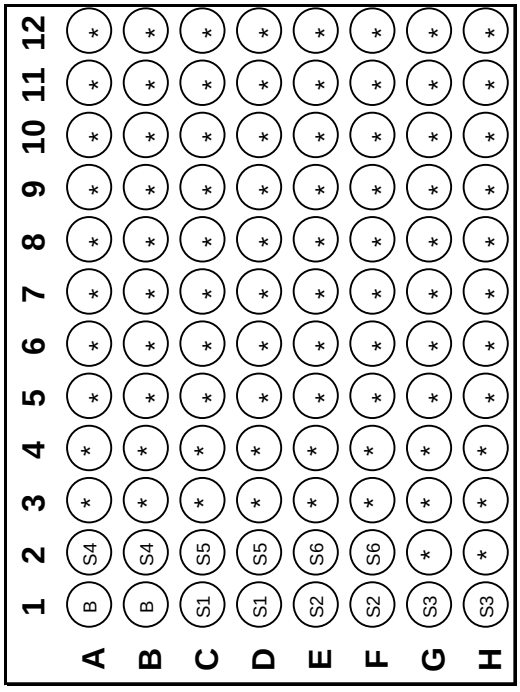
A plate set-up is suggested below. The content of each well may be recorded on the sheet provided with the kit.

PIPETING THE REAGENTS

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

DISTRIBUTION OF REAGENTS AND SAMPLES

- Human Resistin standard:
  Dispense 100 µL of the six diluted standards (S1 to S6) in duplicate to appropriated wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.
- Quality Control and samples:
  Dispense in duplicate 100 µL of diluted Quality Controls, and samples to appropriate wells.
- EIA buffer
  Dispense in duplicate 100 µL to blank (B) wells.



B: Blank
S1-S6: Standards 1-6
*: Samples or Quality Controls

INCUBATING THE PLATE

- Cover the plate with the cover sheet and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- Rinse each well 3 times with Wash buffer (350 µL/well). Slightly shake the plate for 5 minutes (with orbital shaker). Dry by inversion on absorbent paper.

- Biotin labelled anti-Resistin antibody:
  Dispense 100 µL to each well.
- Cover the plate with the cover sheet and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- Rinse each well 3 times with Wash buffer (350 µL/well). Slightly shake the plate for 5 minutes (with orbital shaker). Dry by inversion on absorbent paper.
- Streptavidin-HRP Tracer:
  Dispense 100 µL of tracer to each well.
- Cover the plate with the cover sheet and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- Rinse each well 3 times with Wash buffer (350 µL/well). Slightly shake the plate for 5 minutes (with orbital shaker). Dry by inversion on absorbent paper.

DEVELOPING AND READING THE PLATE

- Dispense 100 µL of Substrate solution to the 96 wells. Incubate the plate in the dark during 10 minutes at room temperature. 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 1 hour			
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 substracted 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 semi-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. Samples with a concentration greater than 50 ng/mL should be re-assayed after dilution.

- 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

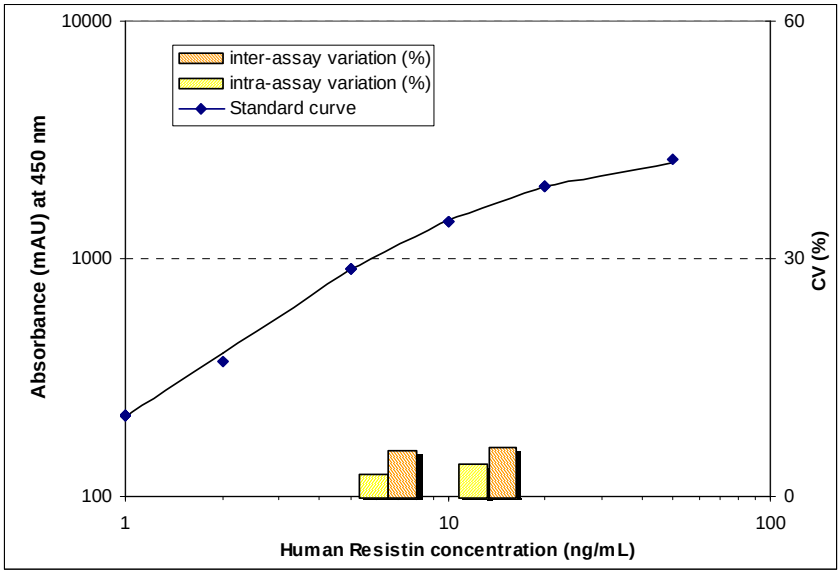
The following data are for demonstration purposes only. Your data may be different but still correct. These data were obtained using all reagents supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Human Resistin	mAU
Standard 50 ng/mL	2 641
Standard 20 ng/mL	2 034
Standard 10 ng/mL	1 423
Standard 5 ng/mL	903
Standard 2 ng/mL	367
Standard 1 ng/mL	218
Blank	5
QC High	2 162
QC Low	916

ACCEPTABLE RANGE

☞ QC samples: see label on the vials.

HUMAN RESISTIN STANDARD CURVE



ASSAY VALIDATION AND CHARACTERISTICS

This assay may be used to measure human Resistin in human samples such as serum, plasma and culture supernatant.

☞ Cross-reactivity:

Horse adiponectin	<0.1%
Human leptin, leptin receptor, adiponectin	<0.1%
Human TNF-α, AFABP, IL-6, AGRP, ASP	<0.1%
Mouse, rabbit, goat, rat, sheep Resistin	<0.1%
Chicken, hamster, and bovine Resistin:	<0.1%
Horse serum equivalent to:	21 ng/mL
Pig serum equivalent to:	12 ng/mL
Monkey serum equivalent to:	6 ng/mL

☞ Sensitivity:

The limit of detection (defined as such a concentration of human AgRP giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + 3*SD_{blank}) is better than 0.1 ng/mL. The EIA buffer was pipetted into blank wells, and the microtiter plate is blanked on air.

☞ Precision:

- Intra-assay variation (n=8)

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1	7.53	0.21	2.8
2	11.35	0.39	3.4

- Inter-assay variation (n=3)

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1	6.46	0.33	5.1
2	13.35	0.93	6.9

☞ Recovery test:

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

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	5.55	-	-
	8.99	10.55	85.2
	13.35	15.55	85.9
	25.34	25.55	99.2
2	7.47	-	-
	10.88	12.47	87.2
	16.79	17.47	96.1
	26.07	27.47	94.9

☞ Dilution test:

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	12.56	-	-
	1:2	6.51	6.28	103.7
	1:4	2.98	3.14	94.9
	1:8	1.74	1.57	110.8
2	-	28.46	-	-
	1:2	14.02	14.23	98.5
	1:4	7.27	7.12	102.1
	1:8	3.88	3.56	108.9

☞ Serum/Plasma sample:
Samples from 16 healthy individuals were taken and treated by different methods, results shown below:

Sample (n=16)	Mean (ng/mL)	Plasma / Serum (%)
Serum	8.13	-
Plasma - Heparin	7.61	99.1
Plasma - Citrate	7.68	90.2
Plasma - EDTA	8.51	104.7

The normal range with serum samples from healthy donors has been established with the human Resistin EIA KIT: 8.1± 4.0 ng/mL (average ± 2 SD, N = 123)

However, it is recommended that each laboratory establishes its own normal range of serum Resistin. The reference range should be regarded as guideline only.

☞ Stability of samples at 4℃:

Samples should be stored at -20℃. However, no decl ine was observed in concentration of human Resistin in serum and plasma samples when stored at 4℃ for 10 days. To avoid microbial contamination, add NaN3 to a final concentration 0.1% to the samples.

Sample	Temp /Time	Serum (ng/mL)	Plasma (ng/mL)		
			Heparin	Citrate	EDTA
1	-	14.1	11.2	12.0	13.3
	4℃/1 day	14.5	13.2	13.1	10.7
	4℃/10 days	14.2	10.9	13.2	12.9
2	-	9.3	8.9	7.8	9.3
	4℃/1 day	10.2	8.4	8.2	9.9
	4℃/10 days	8.8	8.7	7.4	8.7
3	-	5.1	6.0	4.8	5.4
	4℃/1 day	5.1	5.4	4.6	5.7
	4℃/10 days	5.4	5.5	4.1	5.5

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, 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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