

Human Resistin Sandwich ELISA Kit Datasheet

For the quantitative detection of human Resistin concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00159
Product Name	Human Resistin Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	56729
SwissProt	Q9HD89

Kit Components & Storage

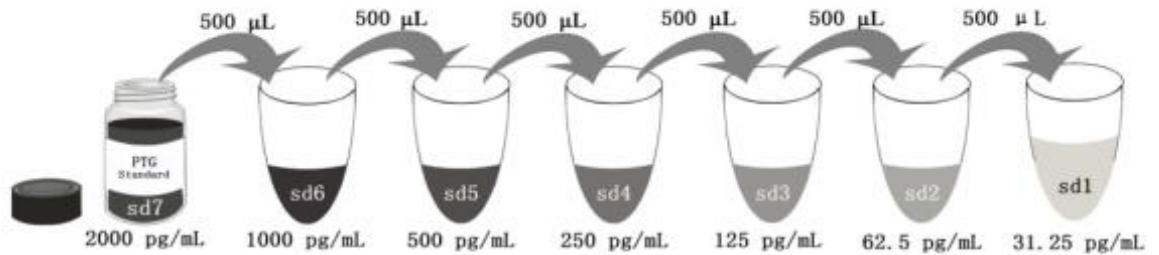
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 2000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-ef is for protein standard and all samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP antibody.

*Add 1 mL Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00159 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Resistin ELISA kit is to be used to detect and quantify protein levels of endogenous Resistin. The assay recognizes human Resistin. An antibody specific for Resistin has been pre-coated onto the microwells. The Resistin protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for Resistin is added to detect the captured Resistin protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Resistin, a cysteine-rich hormone secreted primarily by rodent fat cells, was postulated to be implicated in obesity, insulin resistance and diabetes. Resistin is also secreted by macrophages in humans, promoting inflammation (PMID: 20148674). Resistin modulates the synthesis and secretion of key pro-inflammatory cytokines such as TNF- α , IL-6 and IL12 in macrophages via a nuclear factor-kB (NF-kB)-dependent pathway. Given the connection between inflammation and cancer, resistin was soon considered for its role in cancer. There were observations supporting its possible use as a diagnostic marker, expression levels of which also seemed to differ in patients with varying clinical stages (PMID: 30317640). Resistin has been also linked to other pathologies such as atherosclerosis (PMID: 15710760).

Sample Preparation

The serum, plasma, cell culture supernatants samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

Safety Notes

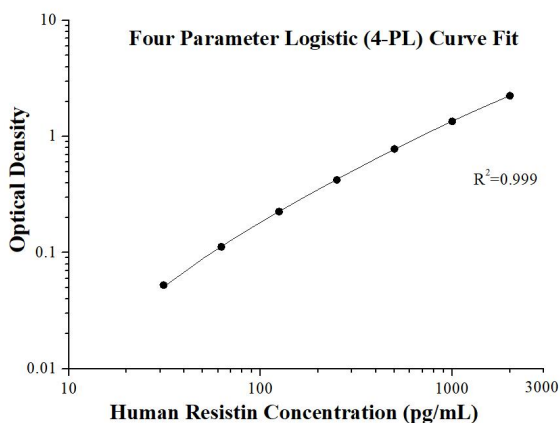
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

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



(pg/mL)	O.D	Average	Corrected
0	0.059 0.057	0.058	-
31.25	0.11 0.111	0.110	0.052
62.5	0.17 0.17	0.17	0.112
125	0.28 0.287	0.284	0.225
250	0.48 0.483	0.481	0.423
500	0.836 0.846	0.841	0.783
1000	1.423 1.398	1.410	1.352
2000	2.332 2.278	2.305	2.247

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	977.2	35.8	3.7	1	24	961.4	47.4	4.9
2	20	216.5	10.9	5.0	2	24	224.2	10.5	4.7
3	20	51.5	3.7	7.2	3	24	52.3	3.3	6.2

Recovery

The recovery of Resistin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average % of Expected	Range (%)
Human plasma	1:8	87	81-92
	1:16	90	79-103
Cell culture supernatants	1:2	84	77-89
	1:4	87	77-96

Sample Values

Serum samples from healthy volunteers were evaluated for Resistin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum(n=16)	3,641	1,306-6,981

Cell culture supernatants-Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μ g/mL streptomycin sulfate. Cells were stimulated with 100 ng/mL LPS. Aliquots of the cell culture supernate were removed on days 5 assayed for levels of human Resistin.

Condition	Day 5 (pg/mL)
Unstimulated	138.8
Stimulated	283.4

Sensitivity

The minimum detectable dose of human Resistin is 15.2 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, plasma samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatants were spiked with high concentrations of Resistin with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	100	93
	Range (%)	-	88-97
1:4	Average% of Expected	113	99
	Range (%)	106-121	91-105
1:8	Average% of Expected	109	100
	Range (%)	103-114	94-105
1:16	Average% of Expected	98	102
	Range (%)	96-100	95-109

References

1. Olefsky JM et al., Macrophages, inflammation and insulin resistance. *Annu Rev Physiol* 2010 72: 219-246.
2. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA & Rader J 2005 Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 111 932-939.
3. Zhang M et al., Resistin effects on pancreatic cancer progression and chemoresistance are mediated through its receptors CAP1 and TLR4. *J Cell Physiol* 2019 234: 9457-9466.