Resistin (human) Serum ELISA Kit

(Catalog #K4919-100; 100 assays; Store kit at 4° C)

I. Description:

Obesity is a well-known risk factor of type 2 diabetes mellitus and is strongly associated with insulin resistance. Resistin (also called FIZZ3/ADSF) is an adipocyte-derived peptide first identified during a search for targets of thiazolidinediones. Serum concentrations of resistin are markedly increased in obese mice and are decreased by treatment with thiazolidinediones. It was also found that administration of an anti-resistin antibody increases insulin-stimulated glucose uptake in obese mice and that treatment of normal mice with recombinant resistin impairs insulin action. Thus, resistin might link obesity with insulin resistance and diabetes in mice models. However, subsequent studies in rodent models have produced disparate findings on the role of resistin in obesity and insulin resistance. In humans, while the expression of resistin in human adipocytes is very low compared with that seen in rodents and does not differ between normal, insulin-resistant or type 2 diabetic individuals, a more recent study using a large size of case suggests that the plasma resistin levels are increased in type 2 diabetes. Therefore determination of the plasma resistin levels may be important for understanding onsets of metabolic diseases such as type 2 diabetes or obesity. This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human resistin in biological fluids. A monoclonal antibody specific for resistin has been precoated onto the 96-well microtiter plate. Standards and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, resistin is recognized by the addition of a biotinylated polyclonal antibody specific for resistin (Detection Antibody). After removal of excess biotinylated antibody, HRP labeled streptavidin (Detector) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of resistin in the samples. The assay range is 0.125–8 ng/ml resistin/ml. The lowest level of resistin that can be detected by this assay is 100 pg/ml.

II. Kit Contents:

Component	100 Assays	Part Number
1 plate coated with human resistin Antibody	(6 x 16-well strips)	K4919-100-1
2 bottle Wash Buffer 10X	(2x30 ml)	K4919-100-2
2 bottle ELISA Buffer 10X	(2x30 ml)	K4919-100-3
1 vial Detection Antibody	(30 µl)	K4919-100-4
1 vial HRP Labeled Streptavidin	2 µg	K4919-100-5
1 vial human resistin Standard (lyophilized)	(16 ng)	K4919-100-6
1 bottle Substrate Solution (TMB)	(12 ml)	K4919-100-7
1 bottle Stop Solution	(12 ml)	K4919-100-8
Plate sealers	2	K4919-100-9

III. Storage Conditions:

Reagents must be stored at 2 - 8°C when not in use. Bring reagents to room temperature before use. Do not expose reagents to temperatures greater than 25°C.

IV. Assay Procedure (Read the ENTIRE Protocol Before Proceeding)

Test Samples/Standards: (We recommend these be run in duplicate)

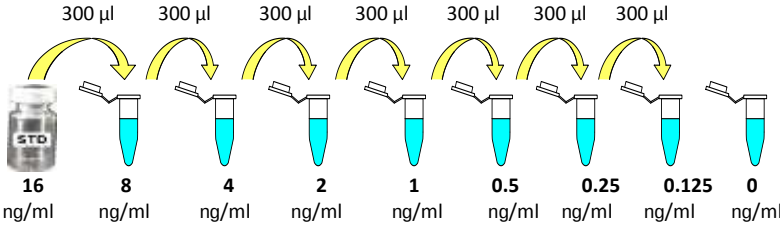
a. **Serum :** Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

b. **Plasma :** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.
NOTE: *Serum, Plasma or Cell Culture Supernatant has to be diluted in* ELISA Buffer 1X. *Samples containing visible precipitates must be clarified before use. As a starting point, 1/20 dilution of serum or plasma is recommended! If samples fall the outside range of assay, a lower or higher dilution may be required!*

c. **Standards**: Reconstitute human resistin Standard with 1 ml of dH_2O to produce a stock solution (16 ng/ml). Mix the Stock solution to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The reconstituted standard should be aliquoted and stored at -20°C.

d) Prepare Standard Curve using 2-fold serial dilutions with 1X ELISA Buffer:

To obtain	Add	Into
8 ng/ml	300 µl of resistin (16 ng/ml)	300 µl of 1X ELISA Buffer
4 ng/ml	300 µl of resistin (8 ng/ml)	300 µl of 1X ELISA Buffer
2 ng/ml	300 µl of resistin (4 ng/ml)	300 µl of 1X ELISA Buffer
1 ng/ml	300 µl of resistin (2 ng/ml)	300 µl of 1X ELISA Buffer
0.5 ng/ml	300 µl of resistin (1 ng/ml)	300 µl of 1X ELISA Buffer
0.25 ng/ml	300 µl of resistin (0.5 ng/ml)	300 µl of 1X ELISA Buffer
0.125 ng/ml	300 µl of resistin (0.25 ng/ml)	300 µl of 1X ELISA Buffer
0 ng/ml	300 µl of Diluent 1X	Empty tube



2. Reagent Preparation: (Prepare just the appropriate amounts for the assay)

a) **1X Wash Buffer:** Dilute 10X Wash Buffer 1: 9 with dH_2O to obtain 1X Wash Buffer.

b) **1X ELISA Buffer:** Dilute 10X ELISA Buffer 1: 9 with dH_2O to obtain 1X ELISA Buffer.

c) **Detection Antibody:** has to be diluted to 1:2000 in ELISA Buffer 1x. Note: *The diluted Detection Antibody is not stable and cannot be stored!*

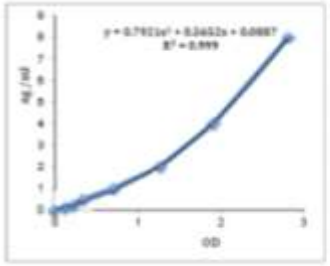
d) **HRP Labeled Streptavidin**: has to be reconstituted with 100 µl of ELISA Buffer 1X. Prepare aliquots and store them at -20°C. **Avoid freeze/thaw cycles**. Dilute the reconstituted STREP-HRP to the working concentration by adding 25 µl in 10 ml of ELISA Buffer 1X (1:400). **Note:** The diluted STREP-HRP is not stable and cannot be stored.

3. Assay Protocol:

a) Determine the number of 16-well strips needed for assay and insert them into the frame for current use. The extra strips should be resealed in the foil pouch and can be stored at 4°C for up to 1 month.
b) Add 100 µl of the Standards, and Samples into the appropriate wells in duplicate.
c) Cover plate with plate sealer and incubate for 1 hr at 37°C.
d) Aspirate and wash x 3 with 300 µl of 1X Wash Buffer.
e) Add 100 µl detection antibody to each well and tap gently on the side of the plate to mix.
f) Cover plate with plate sealer and incubate for 1 hr at 37°C.
g) Aspirate and wash x 3 with 300 µl of 1X Wash Buffer.
h) Add 100 µl of diluted HRP labelled streptavidin into each well.
i) Cover plate with plate sealer and incubate for 1 hr at 37°C.
j) Aspirate and wash x 5 with 300 µl of 1X Wash Buffer.
k) Add 100 µl of TMB substrate solution.
l) Allow the color to develop at room temperature in the dark for 10 min.
m) Stop the reaction by adding 100 µl of Stop Solution to each well.
n) Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added. **Caution: Stop Solution is a Corrosive Solution**
o) Measure the OD at 450 nm in an ELISA plate reader within 30 min.

V. Calculations:

- Average the duplicate readings for each standard, QC and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding resistin concentration (ng/ml) on the vertical (Y) axis (see **10.** TYPICAL DATA).
- Calculate the resistin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human resistin in the samples.



Standard hResistin (ng/ml)	Optical Density (mean)
8	2.816
4	1.913
2	1.2685
1	0.6895
0.5	0.3395
0.25	0.2265
0.125	0.129
0	0

Figure: Standard curve

VI. Performance Characteristics:

1. **Intra-assay precision:** Five samples of known concentrations of human resistin were assayed in replicates 10 times to test precision within an assay.

Samples	Mean	SD	CV (%)	n
1	10.78	0.31	2.86	10
2	19.23	0.99	5.17	10
3	21.49	0.67	3.12	10
4	5.19	0.20	3.77	10
5	12.57	0.47	3.73	10

2. **Inter-assay precision:** Five samples of known concentrations of human resistin were assayed in 10 separate assays to test precision between assays.

Samples	Mean	SD	CV (%)	n
1	6.80	0.49	7.20	10
2	22.96	1.24	5.40	10
3	6.49	0.27	4.20	10
4	15.32	1.07	6.97	10
5	25.66	1.11	4.35	10

3. **Expected values:** Resistin levels range in plasma and serum from **1 to > 20 ng/ml** (from healthy donors).

4. **Recovery:** When samples (serum or plasma) are spiked with known concentrations of human resistin, the recovery averages 96% (range from 93% to 108%).

Samples	Average Recovery (%)	Range (%)
1	94.4	93-96
2	96.6	95-97
3	98.9	92-108

Technical Hints and Limitations:

- It is recommended that all standards, QC sample and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 8-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep Substrate Solution protected from light.
- The Stop Solution consists of sulphuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

Troubleshooting:

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

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