

Human Resistin ELISA Development Kit (Catalog #K4559-100; 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 is an adipocyte-derived peptide first identified during a search for targets of thiazolidinediones. Steppan *et al.* reported that 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. 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. Genetic case-control studies have demonstrated that genetic variations in the resistin gene are associated with insulin resistance and obesity. Therefore determination of the plasma resistin levels may be important for understanding onsets of metabolic diseases such as type 2 diabetes or obesity.

I. Kit Components:

- 1) Antibody coated 96-well plate
- 2) 5X Wash concentrate, 100 ml
- 3) 5X Diluent, 50 ml
- 4) Secondary antibody, 12 ml
- 5) 100X Detector, 150 µl
- 6) Standard, recombinant human resistin, lyophilized
- 7) QC sample= positive control having 12-18 µg/ml range of human plasma adiponectin
- 8) Substrate I, 6 ml
- 9) Substrate II, 6 ml
- 10) Stop solution, 12 ml

II. Storage Conditions:

Reagents must be stored at 2-8°C when not in use. The reagents must be brought up to room temperature before use. Do not expose the reagents to temperature above 25°C. Diluted wash solution may be stored at room temperature for up to one month.

III. Recommended Materials:

- Precision single and multi-channel pipettes
- Disposable pipette tips
- Microtubes or equivalent for preparing dilutions.
- Disposable plastic containers for preparing working detector
- Microplate reader (450 nm)
- Deionized water

III. Assay Procedure

1) Preparation of Reagents

1. Allow all samples and kit components to equilibrate to room temperature (20-25°C).

2. Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use (See table below).

It is recommended that standards and samples be run in duplicate.

3. Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
4. Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
5. Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
6. Freshly prepare just before use the **Substrate Solution** by adding one part Substrate I to one part Substrate II.

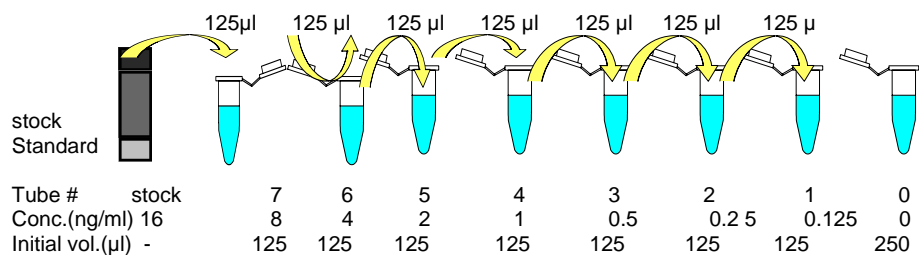
The amount of working reagents needed for 1 well

Working reagents	Total volume needed	Stock solution added	Dilution solution added	Note
1X Wash Solution	2.8 ml	0.56 ml of 5X Wash Concentrate	2.24 ml of ddH ₂ O	Stable for 1 month at RT
1X Diluent	2.4 ml	0.48 ml of 5X Diluent	1.92 ml of ddH ₂ O	in the case of 10 µl sample; Including standard dilution
1X Detector	110 µl	1.1 µl of 100X Detector	108.9 µl of 1X Diluent	Use within 1 hr.
Substrate Solution	110 µl	55 µl of Substrate I	55 µl of Substrate II	Freshly prepared just before use

7. Prepare working aliquots of the Standard as follows :

Briefly centrifuge the Standard vial. When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water the Standard vial to make a stock concentration of 16 ng/ml. Mix well. A recommended dilution scheme is as follows :

- 1) Label 8 microcentrifuge tubes #0-7. Add 125µl and 250µl of the 1X Diluent to the microcentrifuge tubes #1-7 and #0, respectively.
- 2) Add 125 µl of the stock Standard solution to tube #7 and vortex.
This is Standard tube #7 with a concentration of 8 ng/ml.
- 3) Standards #6 to #1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube #0



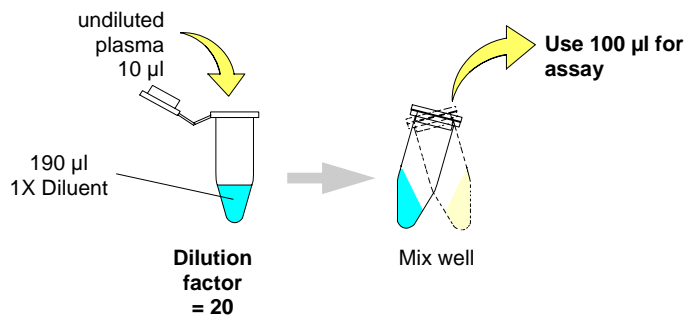
8. Reconstitute QC sample in 1 ml of deionized water.

2) Sample dilution

Step 1. Dilute plasma 1:20 with 1X Diluent (for example, 10 µl sample plus 190 µl 1X Diluent, dilution factor =20) and mix well

Step 2. Use 100 µl of the final diluted plasma for ELISA.

* If samples fall the outside range of assay, a lower or higher dilution may be required..



3) Experiment procedure

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 µl of standards 0 to 7, the reconstituted QC sample and diluted serum sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
3. Incubate at 37°C for 1 hour.
4. Remove the solution and wash 3 times with 250 µl of 1X Wash Solution to each well.
5. Add 100 µl Secondary Antibody to each well.
6. Incubate at 37°C for 1 hour.
7. Remove the solution and wash 3 times with 250 µl of 1X Wash Solution to each well.

8. Add 100µl 1X Detector to each well.
9. Incubate at 37°C for 1 hour.
10. Remove the solution and wash 5 times with 250 µl of 1X Wash Solution to each well.
11. Add 100 µl of the Substrate Solution to each well.
12. Incubate at room temperature for 20 min.

* Protect from light.

13. Using the multi-channel pipette, add 100 µl Stop Solution to each well.
14. Read at 450 nm.
15. Subtract the absorbance of the blank from the readings for each standard and sample.
16. Construct a standard curve by plotting the known concentrations (X) of standard versus the absorbances (Y) of standard. A measurable range is typically shown between 0.125 ng/ml and 1ng/ml.
17. Calculate the adiponectin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
18. The adiponectin concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted samples (Dilution factor of lyophilized QC sample is 20)

