

Human Visfatin ELISA Kit

(Catalog #K4907-100; 100 assays; Store kit at 4°C)

I. Description:

Visfatin, an adipocytokine that is highly enriched in the visceral fat of both humans and mice and whose expression level in plasma increases during the development of obesity. Visfatin corresponds to pre-B cell colony-enhancing factor (PBEF), a 52-kD cytokine expressed in lymphocytes. PBEF is an inflammatory cytokine that plays a requisite role in the delayed neutrophil apoptosis of sepsis. Visfatin exerted insulin-mimetic effects in cultured cells and lowered plasma glucose levels in mice. It was found that visfatin binds to and activates the insulin receptor. Plasma level of visfatin in patients with type 2 diabetes mellitus was elevated, suggesting that measurement of plasma visfatin provides a relevant tool for understanding metabolic diseases.

II. Components:

- 1) Antibody coated 96-well plate (12 x 8 well strips, with absorbed monoclonal antibody to human visfatin)
- 2) 5X Wash concentrate, 100 ml
- 3) 5X Diluent, 50 ml (for reagent dilution)
- 4) 1X Secondary antibody, 12 ml (Rabbit Polyclonal antibody against human visfatin)
- 5) 100X Detector, 150 μ l (HRP conjugated anti-rabbit IgG)
- 6) Standard, recombinant human visfatin (16 ng), lyophilized
- 7) QC sample= 2 positive control of recombinant human visfatin (3 ng/ml, 0.2 ng/ml)
- 8) Substrate, 12 ml (chromogenic reagent)
- 9) Stop solution, 12 ml (1 M H₃PO₄)
- 10) Plate sealer (3 sealers)

III. Storage Conditions:

Reagents must be stored at 2-8°C when not in use. Bring reagents to room temperature before use. Diluted wash solution may be stored at room temperature for up to one month.

IV. Sample Collection and Storage:

Serum: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1000xg. Assay freshly prepared serum or store serum in aliquot at \leq -20°C for future use. Avoid repeated freeze/thaw cycles.

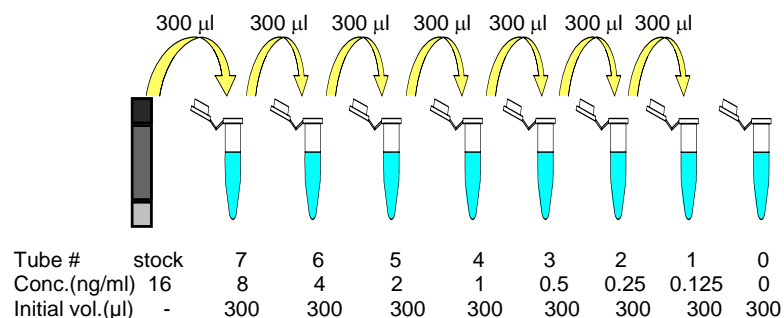
Plasma: We do not recommend use of plasma.

V. 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. 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.
- 5) Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent. Use the 1X Detector within one hour of preparation.

- 6) Warm **Substrate Solution** to room temperature before use.
- 7) Prepare working aliquots of the Standard as follows :
When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping.
- 8) Add 1 ml of deionized water to the Standard vial to make a stock concentration of 16 ng/ml. Mix well. A recommended dilution scheme is as follows:



- a. Label 8 microcentrifuge tubes #0-7. Add 300 μ l of the 1X Diluent to the microcentrifuge tubes #0-7, respectively.
 - b. Add 300 μ l of the stock Standard solution to tube #7 and vortex. This is the Standard tube #7 with a concentration of 8 ng/ μ l.
 - c. Standards #6 to #1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add standard to the tube #0.
- 9) Reconstitute QC sample in 1 ml of deionized water.

2. Experiment procedure

- 1) Remove the appropriate number of microwell strips from the sealed foil pouch.
- 2) Pipette 100 μ l of standards #0 to #7, the reconstituted QC sample and sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
- 3) Incubate at 4°C for overnight.
- 4) Remove the solution and wash 3 times with 300 μ l of 1X Wash Solution to each well.
- 5) Add 100 μ l Secondary Antibody to each well.
- 6) Incubate at 37°C for 1 hour.
- 7) Remove the solution and wash 3 times with 300 μ l of 1X Wash Solution to each well.
- 8) Add 100 μ l 1X Detector to each well.
- 9) Incubate at 37°C for 1 hour.
- 10) Remove the solution and wash 5 times with 300 μ l of 1X Wash Solution to each well.
- 11) Add 100 μ l of the Substrate Solution to each well.
- 12) Incubate at room temperature for 10 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 (Y) of standard vs the absorbances (X) of standard. A measurable range is typically shown between 0.125 ng/ml to 8 ng/ml.
- 17) Calculate the Visfatin concentrations of samples by interpolation of the regression curve formula in a form of a 4-parameter equation.
- 18) The visfatin concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted samples.

Assay Sensitivity: 30 pg/ml

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