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 52kDa 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:

Component	100 Assays	Part Number
96-well plate coated with human Visfatin antibody	6 X 16 well strips	K4907-100-1
10X Wash Buffer	30 ml X 2	K4907-100-2
10X ELISA Buffer	30 ml X 2	K4907-100-3
Detection Antibody	60 µl	K4907-100-4
100X HRP (HRP conjugated anti-rabbit IgG)	150 µl	K4907-100-5
Standard, recombinant human visfatin (lyophilized)	16 ng	K4907-100-6
TMB Substrate, (chromogenic reagent)	12 ml	K4907-100-7
Stop solution	12 ml	K4907-100-8
Plate sealer	2	K4907-100-9
Gel minibag	2	K4907-100-10

III. Storage Conditions:

Reagents must be stored at 2 - 8°C when not in use. Bring reagents to room temperature before use.

IV. Sample Collection and Storage:

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

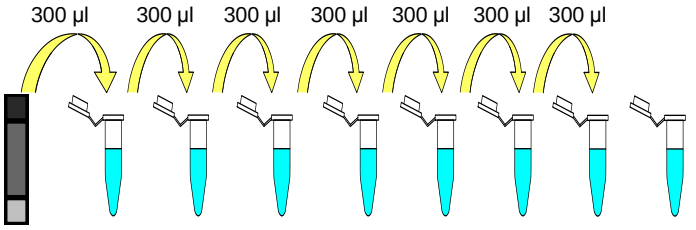
Plasma: We do not recommend use of plasma.

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 10X Wash buffer 1:10 with deionized water before use.
4) Prepare **1X ELISA Buffer**. Dilute 10X ELISA Buffer 1:10 with deionized water before use.
5) Prepare **1X Detection Antibody**. Dilute Detector 1:200 with 1X ELISA Buffer. Use the 1X Detector within one hour of preparation.
6) **HRP 100X (HRP Conjugated anti-rabbit IgG**) has to be diluted to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1X (1:100).
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:



Tube #	stock	7	6	5	4	3	2	1	0
Conc. (ng/ml)	16	8	4	2	1	0.5	0.25	0.125	0
Initial vol. (µl)	-	300	300	300	300	300	300	300	300

a. Label 8 microcentrifuge tubes #0 – 7 and add 300 µl of the 1X ELISA Buffer to each microcentrifuge tube.
b. Add 300 µl of the stock Standard solution to tube #7 and vortex. This is the Standard tube #7 with a concentration of 8 ng/µl.
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 µl of standards and samples 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 300 µl of 1X Wash Solution to each well.
5) Add 100 µl Detection Antibody to each well.

6) Incubate at 37°C for 1 hr.
7) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
8) Add 100 µl 1X HRP Conjugated anti-rabbit IgG to each well.
9) Incubate at 37°C for 1 hr.
10) Remove the solution and wash 5 times with 300 µl of 1X Wash Solution to each well.
11) Add 100 µl of the TMB 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 absorbance at 450 nm within 30 minutes.
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

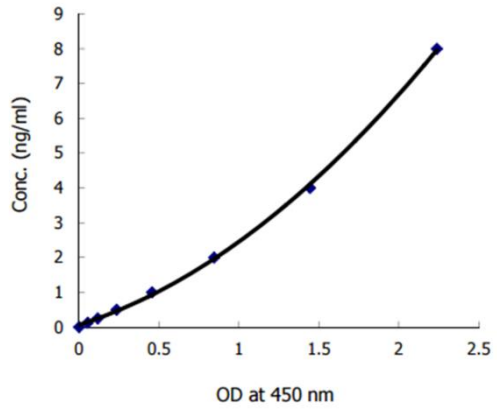


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

FOR RESEARCH USE ONLY! Not to be used on humans.

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- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Set
- Apoptosis siRNA Vectors

Cell Fractionation System
- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence
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- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

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- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

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- cAMP & cGMP Assay Kits
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- CETP Activity Assay & Drug Discovery Kits
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- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays
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