HUMAN VISFATIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN VISFATIN CONCENTRATIONS IN SERUM, AND EDTA PLASMA

VISFATIN IS DETECTABLE IN SALIVA. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY, i.e., WEAR FACE MASK AND GLOVES.

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	**HUMAN VISFATIN ELISA**
Catalog No.	SK00121-09
Lot No.	
Formulation	96 T
Standard range	0.25 - 16 ng/mL
Sensitivity	50 pg/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA Plasma,
Specificity	Human Visfatin
Calibration	Human Visfatin Recombinant
Intra-assay Precision	2 - 5%
Inter-assay Precision	4 - 8%
Storage	2 – 8°C for 6 months. See page 2 for more information
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Visfatin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Visfatin from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Visfatin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Visfatin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human Visfatin. The capture antibody can bind to the human Visfatin in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human Visfatin is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human Visfatin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Visfatin Microplate - 96 well polystyrene microplate coated with an antibody against human Visfatin.	**121-09-01**	**1 plate**
Visfatin Standard – 64 ng/vial of recombinant human soluble Visfatin in a buffered protein base with preservative; lyophilized.	**121-09-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated antibody against human Visfatin with preservative; lyophilized.	**121-09-03**	**1 vial**
Positive Control – one vial of recombinant human soluble Visfatin; lyophilized.	**121-09-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution -11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8°C for up to 6 months. For longer storage for up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20°C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be

stored only at 2 – 8°C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (200 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Human Serum may require 4 ~ 8 dilution.
Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

Dilution Buffer (DB06) - ***If Dilution Buffer is highly viscous, warm in 27 - 30° C water bath until liquid flows more freely.***

Visfatin Standard - Reconstitute the Visfatin standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 64 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of Dilution Buffer into tubes #1 - 4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **16 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	**128 ng/ml**
# 1	100µl of stock	300µl	**16 ng/ml**
# 2	100µl of 1	300µl	**4 ng/ml**
# 3	100µl of 2	300µl	**1 ng/ml**
# 4	100µl of 3	300µl	**0.25 ng/ml**

Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated **Streptavidin-HRP Conjugate** stock solution to 11.88 mL of **HRP Diluent Solution (DB01)** to prepare working solution. ***Note:*** *1x working solution of Streptavidin-HRP Conjugate should be used within 10 min (protect from light).*

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples

should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells.
4. Add 100 µL of **Standard dilutions** in reverse order of serial dilution, **samples**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker (350 -400 rpm) at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 10-15 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve data is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

VISFATIN (NG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.094)
0.25	0.039
1	0.159
4	0.617
16	2.206

- Lot No.:
- Positive Control: lot specifc

LINEARITY

To assess the linearity of the assay, pooled human serum samples were diluted with Dilution Buffer (DB06) and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
2 x	10.102	20.204	100
4 x	5.031	20.124	99

To assess the linearity of the assay, pooled research human EDTA plasma samples were diluted with Dilution Buffer (DB06) and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1 x	1.412	1.412	100
2 x	0.795	1.591	113

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Soluble Visfatin	100%
Rat Soluble Visfatin	0
Mouse Soluble Visfatin	0
Human Adiponectin	0
Human Irisin	0
Human Asprosin	0
Human FGF-21	0
Human Omentin 1	0
Human Endotrophin	0
Human CTRP15	0
Human Resistin	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µL of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µL Detection Antibody working solution to each well. Incubate 90 minutes on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µL streptavidin HRP conjugate working solution to each well. Incubate 45 minutes on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µL Substrate Solution to each well. Incubate 10-15 min on the plate shaker at RT. **Protect from light.**
⬇
Add 100 µL Stop Solution to each well. Read 450nm within 15 min.