RAT VISFATIN ELISA KIT

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

PURCHASE INFORMATION:

ELISA NAME	RAT VISFATIN ELISA
Catalog No.	SK00121-02
Lot No.	
Formulation	96 T
Standard range	1-64 ng/ml
Sensitivity	0.5 ng/ml
Sample Volume	100 µl
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma
Specificity	Rat and Human Visfatin
Intra-assay Precision	6-8%
Inter-assay Precision	10-12%
Storage	2-8°C

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

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INTRODUCTION

Rat Visfatin immunoassay is a solid phase ELISA designed to measure Visfatin in serum and EDTA plasma. It contains recombinant Visfatin and antibodies raised against this protein. It has been shown to accurately quantify recombinant Visfatin. Results obtained with naturally occurring Visfatin samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural rat Visfatin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Visfatin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Visfatin present is bound by the immobilized antibody. After washing away any unbound substances, an antibody specific for Visfatin is added to the wells. Following a wash to remove any unbound antibody reagent, Anti Rabbit IgG HRP Conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of Visfatin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute 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.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Visfatin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified IgG against Visfatin.	**121-02-01**	**1 plate**
Visfatin Standard – 64 ng/vial of recombinant soluble Visfatin in a buffered protein base with preservative; lyophilized.	**121-02-02**	**1 vial**
Detection Antibody Concentrate – 1.05mL/vial, 10-fold concentrate of purified IgG against Visfatin with preservative; lyophilized.	**121-02-03**	**1 vial**
Positive Control – one vial of recombinant soluble Visfatin, lyophilized.	**121-02-04**	**1 vial**
Anti Rabbit IgG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Anti Rabbit IgG conjugate to HRP	**ARIGHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
ARIGHRP Diluent Solution - 12mL of buffered protein based solution with preservative. Ready to use.	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-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 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control, and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. ARIGHRP Conjugate 100-fold

concentrate (protect from light) and other components may be stored at 2 - 8° C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 month at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples do not require dilution.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

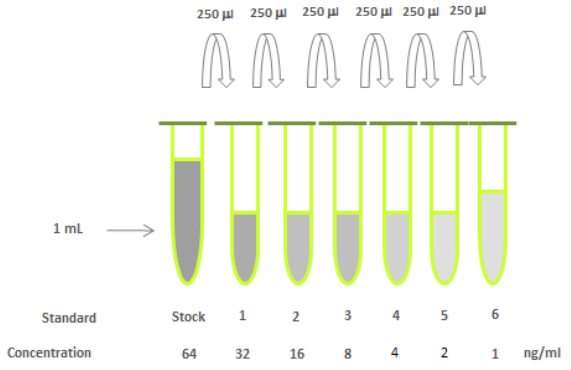
PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.
Visfatin Standard - Refer to vial label for reconstitution volume. Reconstitute the **VISFATIN** standard with 1.0 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 250 µL of Dilution Buffer into tubes #1-6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 64 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	64 ng/ml
# 1	250µl of stock	250µl	32 ng/ml
# 2	250µl of 1	250µl	16 ng/ml
# 3	250µl of 2	250µl	8 ng/ml
# 4	250µl of 3	250µl	4 ng/ml
# 5	250µl of 4	250µl	2 ng/ml
# 6	250µl of 5	250µl	1 ng/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 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.

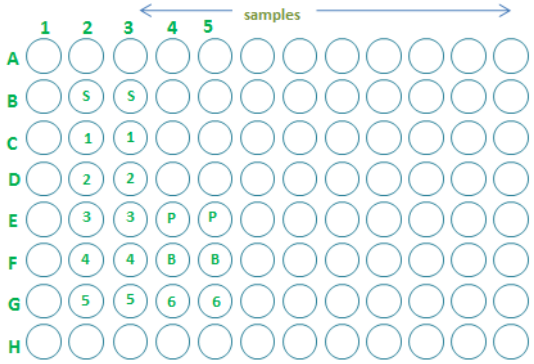
Anti Rabbit IgG HRP Conjugate - Transfer 120 µl of 100-fold concentrated **Anti Rabbit IgG HRP Conjugate** stock solution to 11.88 mL of **ARIGHRP Diluent Solution** to prepare working solution. *Note: 1x working solution of Anti Rabbit IgG-HRP Conjugate should be used within a few days (protect from light).*

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. *Note: Positive Control should be prepared and used within a few days if stored at -20 °C or -70°C.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (F4, F5).
4. Add 100 µL of Standard solutions in reverse order of serial dilution (from G4, G5 and G2, G3 to B2, B3), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker (250-300rpm) at room temperature. A plate layout is provided to record standards and samples assayed.
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 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Anti Rabbit IgG-HRP Conjugate** working solution to each well. Incubate for 1 hour 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 25-35 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

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Visfatin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant soluble Visfatin.

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 OD450 (CORRECTED)
Blank	0 (0.069)
1	0.030
2	0.064
4	0.122
8	0.177
16	0.327
32	0.605
64	0.884

- **Lot No.:**
- **Positive Control:**

SENSITIVITY

The minimum detectable dose (MDD) of Visfatin was 0.5 ng/mL.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Rat Soluble Visfatin	100
Human Soluble Visfatin	100
Human Adiponectin	0
Human Vaspin	0
Human FGF-21	0
Human Omentin 1	0
Human FABP-4	0
Human FTO	0
Human Resistin	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard, samples, 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 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Anti Rabbit IgG HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 25-35 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 min.

REFERENCES

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3. Sun Q, Li L, Li R, Yang M, Liu H, Nowicki MJ, Zong H, Xu J, Yang G. Overexpression of visfatin/PBEF/Nampt alters whole-body insulin sensitivity and lipid profile in rats. Ann Med. 2009;41(4):311-20 .