BioAim Scientific Inc

Mouse Visfatin EasyTest™ Competitive ELISA Kit

Cat.No: 2020003

Instruction Manual (Last revised Sept 20, 2015)

For research use only

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I. INTRODUCTION

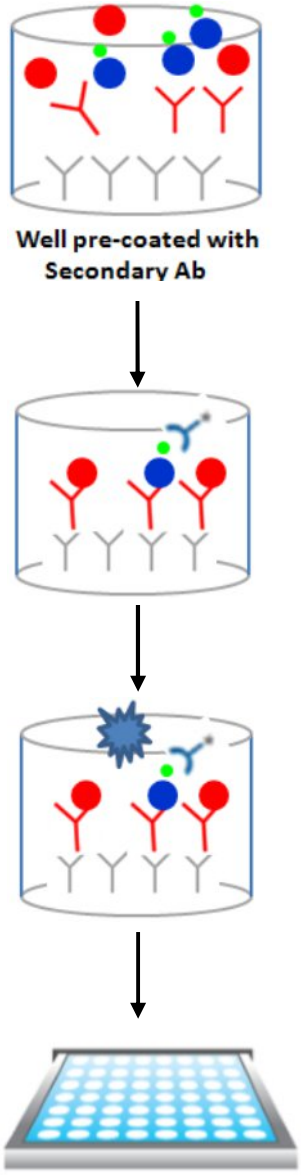
Visfatin (also known as PBEF and Namp) is an enzyme that in mouses is encoded by the PBEF1 gene. This protein has also been reported to be a cytokine (PBEF) that promotes B cell maturation and inhibits neutrophil apoptosis.

It is downregulated by an increase of miR-34a in obesity via a 3'UTR functional binding site of NAMPT mRNA resulting in a reduction of NAD (+) and decreased SIRT1 activity.

Visfatin catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide, one step in the biosynthesis of nicotinamide adenine dinucleotide. The protein is an adipokine that is localized to the bloodstream and has various functions, including the promotion of vascular smooth muscle cell maturation and inhibition of neutrophil apoptosis. It also activates insulin receptor and has insulin-mimetic effects, lowering blood glucose and improving insulin sensitivity. The protein is highly expressed in visceral fat and serum levels of the protein correlate with obesity.

The BioAim mouse Visfatin EasyTest^{TM} Competitive ELISA kit can quantitatively measure Visfatin in mouse serum or plasma. Other matrices, such as urine and tissue, may be suitable but have not been validated. It is a simple and rapid technology for the quantitation of antigen in a range of sample matrices. The whole process takes 2 hours with high accuracy and precision.

II. PRINCIPLE



1. Microplate was pre-coated with secondary Ab;
2. Add Anti-Visfatin Ab first, then sample/Standard and biotinylated peptide;
3. Incubate 1.5h at room temperature.

1. Wash;
2. Add Streptavidin-HRP;
3. Incubate 45min at room temperature.

1. Wash;
2. Add TMB substrate;
3. Incubate 15 min at room temperature.

1. Add stop solution;
2. Read plate at 450nm.

= Anti-rabbit IgG
= Primary antibody
= Sample /standard peptide
= Biotinylated peptide
= Streptavidin -HRP
= TMB substrate

III. MATERIALS SUPPLIED

1. Mouse Visfatin Microplate: 96 wells (12 strips x 8 wells) coated with secondary antibody.
2. Anti-Visfatin antibody: 6µl/vial, 2 vials.
3. Standard Visfatin peptide: 10µl/vial, 2 vials.
4. Biotinylated Visfatin peptide: 10µl/vial, 2 vials.
5. HRP-Streptavidin concentrate: 60 µl 200x concentrated HRP-conjugated Streptavin.
6. 5x Assay Diluent: 1vial, 15 ml.
7. 20x Wash Buffer Concentrate: 1 Vial, 25 ml.
8. TMB Substrate: 12 ml.
9. Stop Solution: 8 ml of 0.2 M sulfuric acid.
10. User Manual.
11. Plate sealer: 2 pieces.

IV. STORAGE

1. The kit can be stored for up to 6 months at 2º to 8ºC from the date of shipment.
2. Standard can be stored at -20 ºC or -80 ºC. Use freshly prepared standard within 12 hours (stored at 2~8ºC).
3. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8ºC. Return unused strip to the pouch containing desiccant pack, reseal along entire edge and keep in 2~8ºC.
4. Avoid repeated freeze-thaw cycles.

V. ADDITIONAL MATERIALS REQUIRED

1. Distilled or deionized water.
2. Precision pipettes, with disposable plastic tips.
3. Beakers, flasks, cylinders necessary for preparation of reagents.
4. Microplate washing device (multichannel pipette or automated microplate washer).

5. Microplate shaker.
6. Microplate reader capable of reading at 450 nm.
7. Materials used for sample preparation.

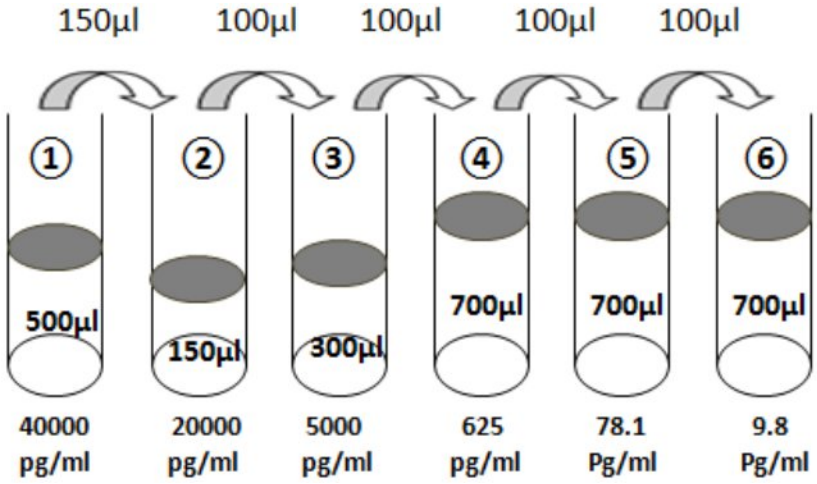
VI. PRECAUTIONS

1. All reagents must be at room temperature (18ºC to 25ºC) before running assay.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.
4. Do not expose kit reagents to strong light during storage or incubation.
5. Use disposable pipette tips for each transfer to avoid microbial contamination or cross contamination of reagents.
6. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results.
7. Avoid contact of stop solution with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
8. Do not use TMB substrate solution if it has begun to turn blue.
9. Do not expose bleach to work area during actual test procedure because of potential interference with enzyme activity.

VII. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18~25°C) before use.
2. **Assay diluent**
   Dilute the concentrated assay diluent 1:5 with distilled water (e.g. 10ml plus 40ml).
3. **Wash buffer**
   Dilute the concentrated wash buffer 1:20 with distilled water (e.g. 20ml plus 380ml).

4. **Anti-Visfatin antibody**
   Briefly centrifuge the vial before use. Add 1494 µl 1x assay diluent to the vial. Pipette up and down to mix thoroughly. A vial can be used for around 60 wells.
5. **Standard Visfatin peptide**
   a. Briefly spin the standard vial before use. Add 490 µl 1x Assay Diluent to prepare a 40ng/ml standard solution. Gently vortex to mix. This is standard #1.
   b. Label 5 tubes #2 through #6. Pipette 150 µl 1x assay diluent into tube #2, pipette 300 µl 1x assay buffer into tube #3. Pipette 700 µl 1x assay diluent into tubes #4 through #6.
   c. Take 150 µl from standard #1 and add to tube #2. Mix thoroughly. Add 100 µl from tube #2 to tube #3. Mix thoroughly. Continue this for tubes #4 through #6.



   The concentrations of Visfatin in the tubes are labeled above. Diluted standards should be used within 60 minutes of preparation.
6. **Biotinylated Visfatin peptide**
   Briefly centrifuge the vial before use. Add 1490 µl of 1x assay diluent to make the final concentration enough for 60 wells.
7. **Streptavidin-HRP**
   The HRP-Streptavidin concentrate should be diluted 200- fold with 1x assay diluent. Do not store diluted Streptavidin-HRP.

VIII. ASSAY PROCEDURE

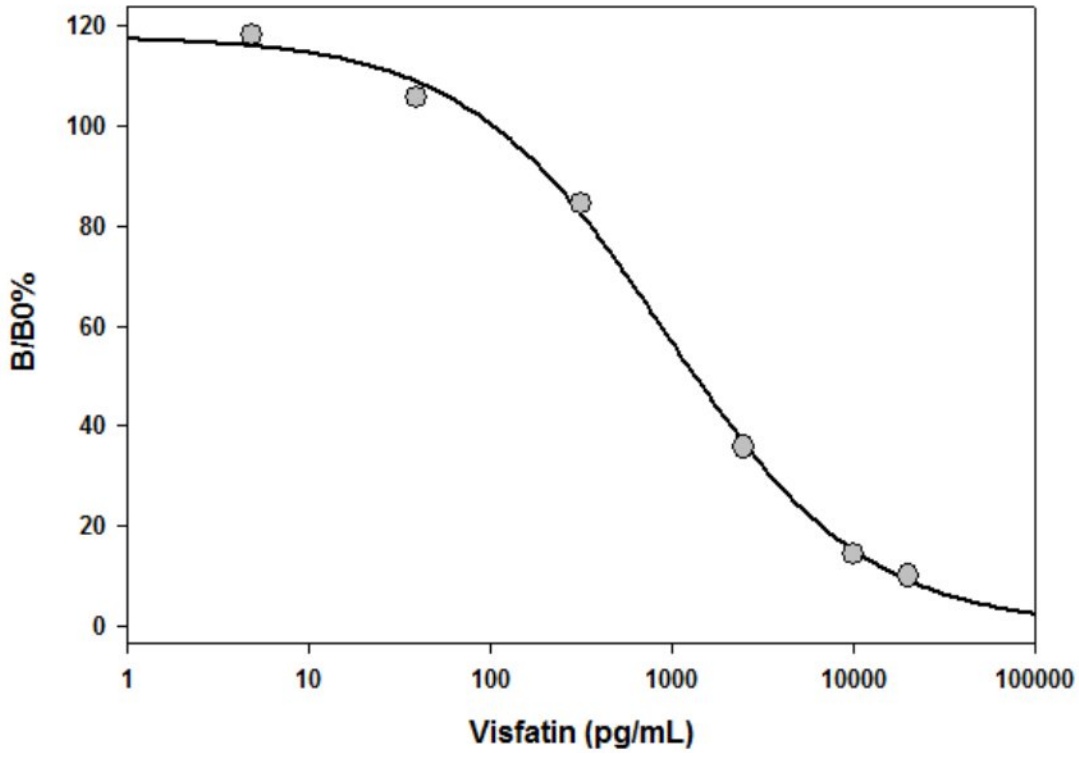
1. All reagents must be brought to room temperature (18-25°C) prior to use. Place the required number of microwells in the holder. It is recommended that all samples, standards, and blanks be run in duplicate.
2. Pipette 25 µl of anti-Visfatin antibody into all wells.
3. Pipette 75 ul of 1x assay diluent into the blank wells.
4. Pipette 50 µl of 1x assay diluent into the Bo (0 ng/ml standard) wells.
5. Pipette 50 µl of Standards #1 through #6 or from #6 to #1 (in reverse order of serial dilution) to the appropriate wells.
6. Pipette 50 µl of samples to the appropriate wells.
7. Pipette 25 µl of biotinylated peptide into each well except the Blank wells.
8. Seal the plate. Incubate for 1.5 hour at room temperature with gentle shaking.
9. Empty the contents of the wells and wash by adding 300 µl of wash buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
10. Pipette 100 µl of diluted streptavidin-HRP solution to each well.
11. Seal the plate. Incubate for 45 min at room temperature with gentle shaking.
12. Wash plate as above (Step 9).
13. Pipette 100 µl of TMB Substrate Solution into each well. Incubate plate for 15 minutes at room temperature in the dark with gentle shaking.
14. Add 50 µl of stop solution to each well.
15. Read absorbance at 450nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570nm from readings at 450nm.

IX. CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of duplicate standards, samples and controls. Subtract the average blank well optical density.
2. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

   Percentage absorbance = (B – blank OD)/ (Bo – blank OD) *100

   Where B = OD of sample or standard and Bo = OD of zero Standard, total binding.
3. A representative standard curve is shown below. This standard curve is for demonstration only. A standard curve must be run with each assay by operator.



X. PERFORMANCE

A. Sensitivity

The minimum detectable dose of Visfatin was determined to be 130pg/ml. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standards.

B. Specificity

No cross-reactivity was identified with the following peptides: Vasopressin, Oxytocin, VIP, Leptin, Adiponectin, Insulin and Resistin.

C. Reproducibility

Intra-Assay CV%: <10%
Inter-Assay CV%: <15%

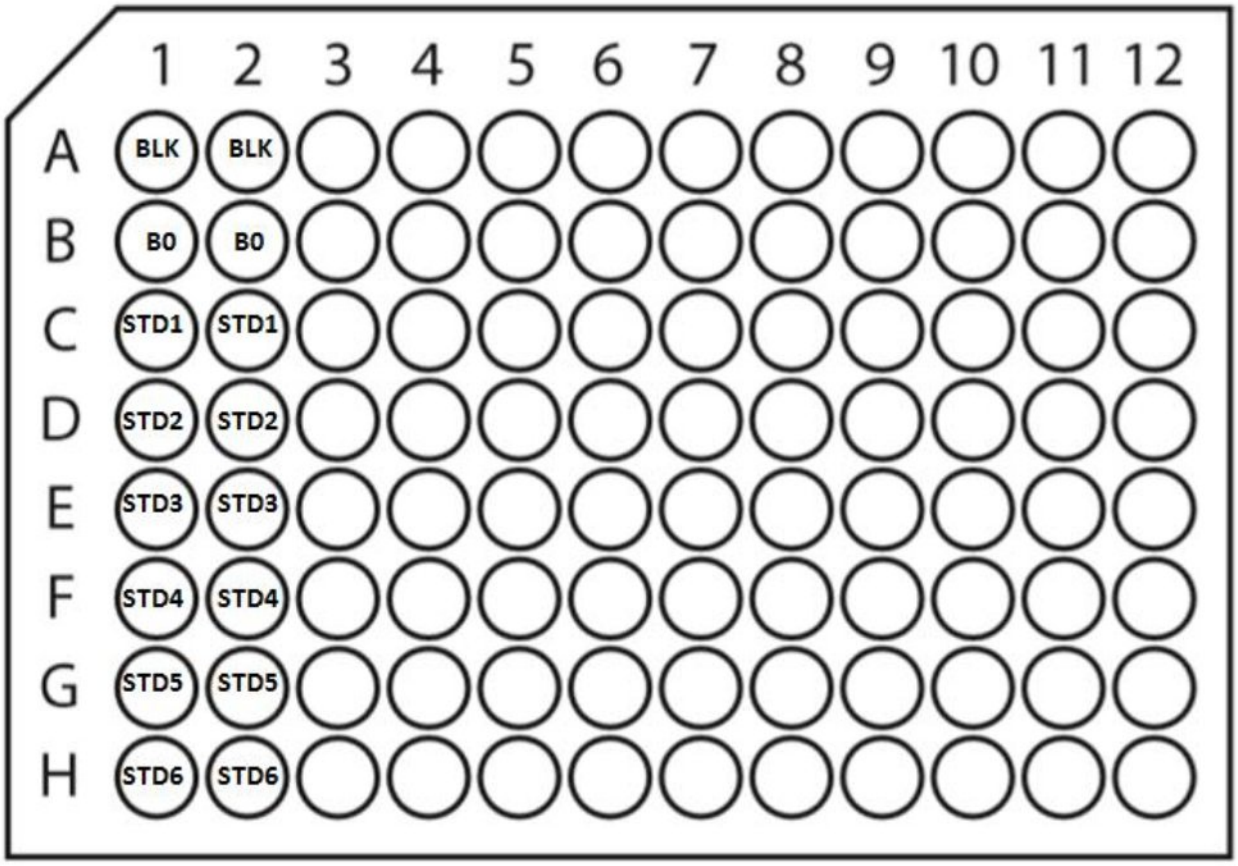
XI. REFERENCES

1. Samal B, etal. (February 1994). Mol. Cell. Biol. 14 (2): 1431–7.
2. Choi SE, etal. (December 2013). Aging Cell. 12 (6): 1062–72.
3. Rongvaux A, etal. (November 2002). Eur. J. Immunol. 32 (11): 3225–34.
4. Wang T, etal. (July 2006). Nat. Struct. Mol. Biol. 13 (7): 661–2.

XII. TROUBLESHOOTING

Problem	Cause	Solution
1.Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. check pipettes; 2. Ensure briefly spin the vial of standard, take the right amount to dilution.
2. Low signal	1. Too brief incubation time 2. Inadequate reagent volumes or improper dilution	1. ensure adequate incubation time; 2. Check pipettes and ensure corrected preparation.
3. Large CV	Inaccurate pipetting	1. Check pipettes; 2. Accurately perform each step.
4.High background	1. Plate is insufficiently washed; 2. Wash buffer contamination	1.Follow the manual correctly; if using a plate washer, check that all ports are working functionally; 2. Prepare fresh buffer.
5. Low sensitivity	1.EIA kit improper storage 2. Stop solution	1. Follow the manual to store each component correctly; 2. Add enough stop solution to each well.

XIII. ASSAY DIAGRAM



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