Product Manual

Nampt (Visfatin/PBEF) (mouse/rat) Dual ELISA Kit

Catalog #: AG-45A-0007EK

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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INTRODUCTION

Fukuhara et al. (1) isolated 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. The gene encoding PBEF was originally isolated from an activated lymphocyte cDNA library (2). Although PBEF lacks a typical signal sequence for secretion, transfected COS-7 and mouse embryonic fibroblasts secreted PBEF into the culture medium. Samal et al. (2) found that recombinant PBEF secreted from transfected COS-7 and mouse embryonic fibroblasts was not itself active in a pre-B-cell colony formation assay, but it synergized the pre-B-cell colony formation activity of stem cell factor and interleukin-7. Jia et al. (3) found that 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. Mice heterozygous for a targeted mutation in the visfatin gene had modestly higher levels of plasma glucose relative to wild type littermates. Surprisingly, it was found that visfatin binds to and activates the insulin receptor (1). However, this original discovery has not been reproduced by two groups (4-5). Visfatin, which is a secretory form of Nampt (nicotinamide phosphoribosyl -transferase), the rate-limiting enzyme of the mammalian NAD, plays a key role in secretion of insulin in the pancreatic beta-cells (5). Recently, two recent studies showed that plasma or serum levels of visfatin in patients with type 2 diabetes mellitus was elevated (6-7), suggesting that measurement of plasma visfatin provides a relevant tool for understanding metabolic diseases.

ASSAY PRINCIPLES

This kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of visfatin in mouse or rat serum. A monoclonal antibody specific for mouse visfatin has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any visfatin present is bound by immobilized antibody. Bound visfatin is captured by purified anti-mouse visfatin polyclonal antibody. HRP conjugated anti- IgG is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded visfatin quantity. The color development is stopped and the intensity of color is measured.

MATERIALS SUPPLIED

1) Antibody coated 96-well plate, 12X 8-well strips
2) 10X Wash concentrate, 2 x 30 mL
3) 10X Diluent, 2 x 30 mL
4) Secondary antibody, 50 µL
5) 100X Detector, 150 µL
6) Standard, recombinant mouse visfatin expressed by HEK 293 cells, 1 vial, lyophilized
7) Substrate, 12 mL
8) Stop solution, 12 mL
9) Plate sealer, 2 sealers

REAGENTS DESCRIPTION

- **Antibody coated 96-well plate**, 12X 8-well strips, with absorbed monoclonal antibody against mouse visfatin
- **10X Wash concentrate**, buffered detergent solution, supplied as a 10X concentrate
- **5X Diluent**, for sample and reagent dilution
- **1X Secondary antibody**, polyclonal antibody against mouse visfatin
- **100X detector**, HRP conjugated anti-IgG
- **Standard**, 64.0 ng, recombinant mouse visfatin
- **Substrate solution**, chromogenic reagents
- **Stop solution**, 1M H3PO4

Reagants require separate storage conditions.

STORAGE

Reagents must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

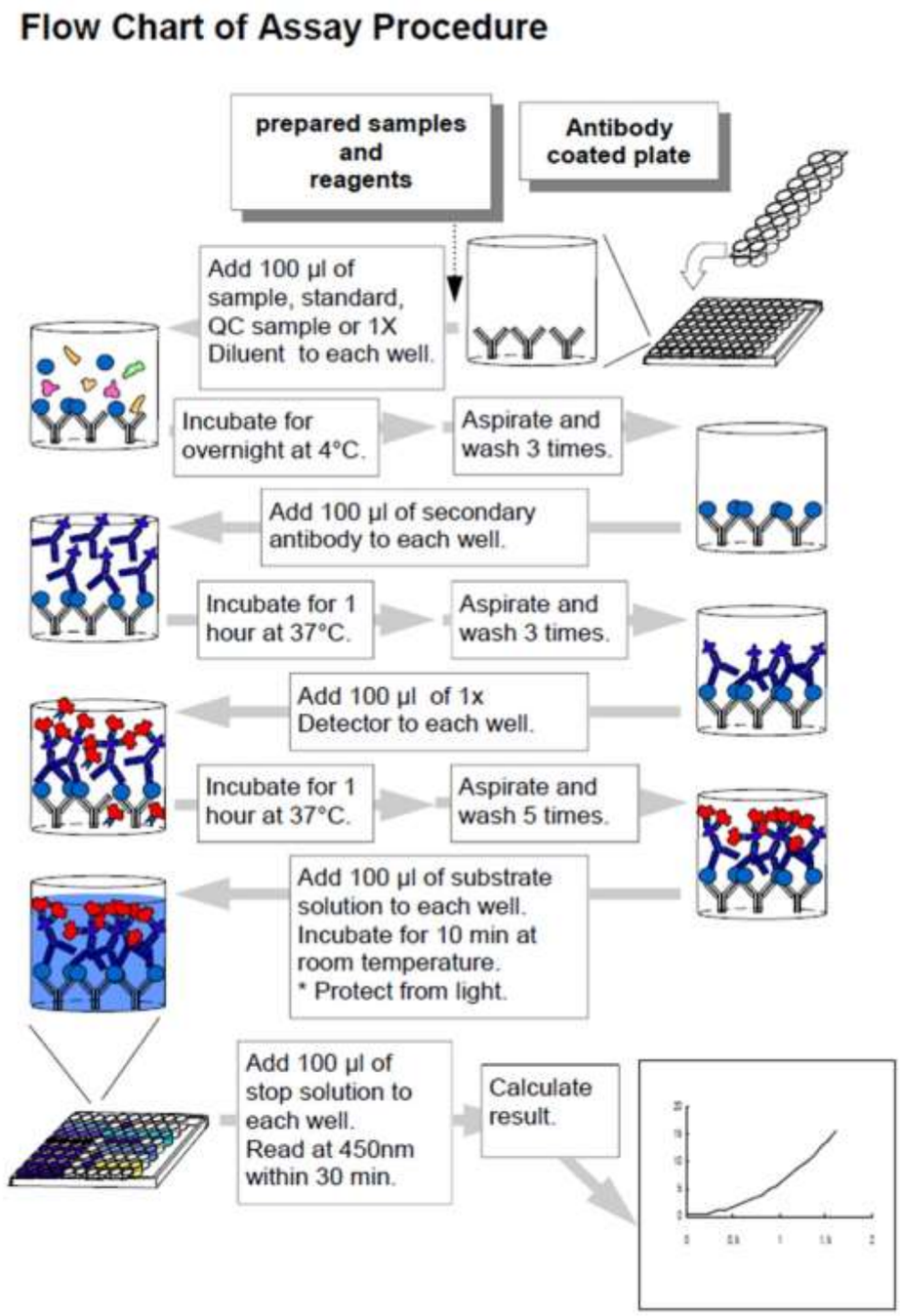
OTHER MATERIALS NEEDED

1. Precision single and multi-channel pipettes
2. Disposable pipette tips
3. Microtubes or equivalent for preparing dilutions
4. Disposable plastic containers for preparing working reagents
5. Reagent reservoirs
6. Microwell or microstrip plate reader 450 nm
7. Deionized water

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 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ - 20oC for later use. Avoid repeated freeze/thaw cycles.

ASSAY PROCEDURE

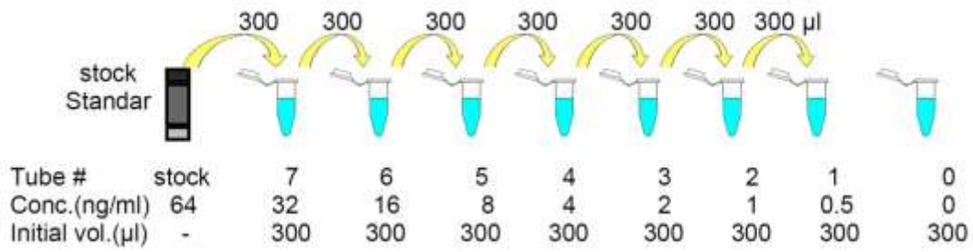


REAGENT PREPARATION

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. It is recommended that standards and samples be run in duplicate.
3. Prepare 1X Wash Solution. Dilute 10X Wash Concentrate 1:10 with deionized water (1 part 10X Wash Concentrate with 9 parts 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 (1 part 5X Diluent with 4 parts deionized water).
5. Prepare Secondary Antibody. Dilute 1:250 in 1X Diluent (1 part Secondary Antibody with 249 parts 1X Diluent). Use the diluted Secondary Antibody within one hour of preparation.
6. Prepare 1X Detector. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
7. Warm Substrate Solution to room temperature before use.
8. 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.
   - Add 1mL of deionized water to the Standard vial to make a stock concentration of 64 ng/mL. Mix well.

A recommended dilution scheme is as follows:

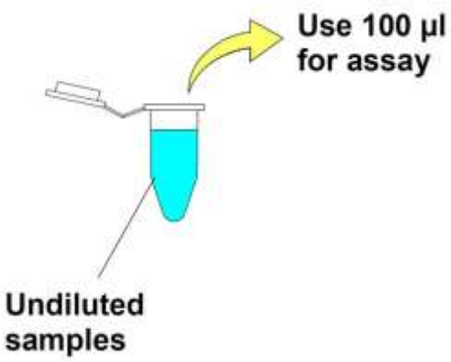
1. Label 8 microcentrifuge tubes #0-7. Add 300 µL of the 1X Diluent to the microcentrifuge tubes #0-7, respectively.
2. Add 300 µL of the stock Standard solution to tube #7 and vortex. This is Standard tube #7 with a concentration of 32 ng/ml.
3. Standards #6 to #1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube #0.



SAMPLE PREPARATION

- Use 100 µL of the undiluted sample for ELISA.

* If samples fall the outside range of assay, a lower or higher dilution may be required.

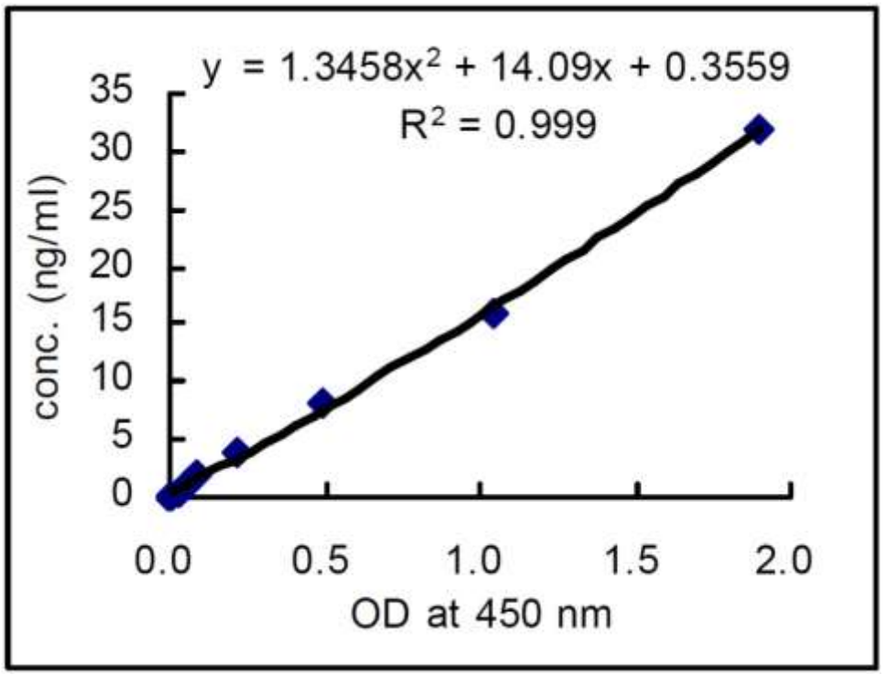


EXPERIMENTAL PROCEDURE

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 µL of standards #0 to #7 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 µL of 1X Wash Solution to each well.
5. Add 100 µL Secondary Antibody to each well.
6. Incubate at 37°C for 1 hour.
7. Remove the solution and wash 3 times with 300 µL of 1X Wash Solution to each well.
8. Add 100 µL 1X Detector to each well.
9. Incubate at 37°C for 1 hour.
10. Remove the solution and wash 5 times with 300 µL of 1X Wash Solution to each well.
11. Add 100 µL 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 450nm.
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 versus the absorbance (X) of standard. A measurable range is typically shown between 0.5 ng/mL and 32 ng/mL.
17. Calculate the visfatin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
18. The visfatin concentrations calculated must be multiplied by dilution factor [see **Sample Preparation**] to obtain the concentrations of the undiluted samples.

STANDARD CURVE



PERFORMANCE CHARACTERISTICS

Sensitivity

50 pg/mL

Precision

Intra-assay precision (precision within an assay)

6 samples were tested 5 times to assess intra-assay precision.

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
Mouse serum 1	5.66	0.30	5.30
Mouse serum 2	16.70	1.04	6.21
Mouse serum 3	9.65	0.43	4.44
Rat serum 1	13.59	0.06	0.41
Rat serum 2	10.61	0.17	1.64
Rat serum 3	19.75	0.36	1.84

Inter-assay precision (precision between assays)

6 samples were tested 6 times to assess inter-assay precision.

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
Mouse serum 1	8.20	0.72	8.80
Mouse serum 2	7.93	0.75	9.44
Mouse serum 3	12.62	0.84	6.68
Rat serum 1	10.75	0.29	2.69
Rat serum 2	19.39	0.91	4.71
Rat serum 3	8.99	0.44	4.87

Specificity

Cross Reactivities

Cross reactivity of a given analyte at 10 ng/mL or 100 ng/mL was calculated as an optical density in relative to 10 ng/mL of recombinant mouse visfatin.

Analyte (recombinant proteins)	Max. Conc. (ng/ml)	Cross Reactivity (%)
Mouse Visfatin	10	100
Rat Visfatin	10	100
Human Visfatin	100	N. R.
Mouse Adiponectin	100	N. R.
Mouse Resistin	100	N. R.
Mouse Vaspin	100	N. R.
Mouse RBP4	100	N. R.
Mouse GPX3	100	N. R.
Mouse Progranulin	100	N. R.
Mouse IL-33	100	N. R.
Mouse Clusterin	100	N. R.
Mouse ANGPTL3	100	N. R.
Mouse ANGPTL4	100	N. R.
Human AGF	100	N. R.
Mouse RBP4	100	N. R.
Mouse Leptin	100	N. R.
Mouse TNF-α	100	N. R.
Human adiponectin	100	N. R.

N. R: No Cross-reactivity

Recovery

The recovery of visfatin spiked to three different levels in four different samples throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
Mouse serum 1	100.70	95-105
Mouse serum 2	99.45	90-100
Rat serum 1	100.70	95-105
Rat serum 2	104.65	95-105

Linearity - Effect of Serum Dilution

To assess the linearity of the assay, four serum samples were first diluted as indicated below prior to sample preparation as described in the protocol.

Sample No.	Serum Dilution	Expected (ng/mL)	Observed (ng/mL)	% of Expected
Mouse serum 1	1	6.02	6.02	100
	½	3.01	3.07	101.98
	¼	1.50	1.40	93.27
Mouse serum 2	1	4.57	4.57	100
	½	2.29	1.90	83.24
	¼	1.14	1.23	107.86
Rat serum 1	1	6.53	6.53	100
	½	3.27	3.38	103.33
	¼	1.63	1.52	92.90
Rat serum 2	1	9.64	9.64	100
	½	4.82	4.60	95.42
	¼	2.41	2.34	97.11

% of expected = observed / expected x 100%

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PLATE LAYOUT

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
1								
1								
1								

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

NOTES

NOTES

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