ab285348– Human Visfatin Intracellular ELISA Kit

For the quantitative measurement of human Visfatin.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285348

Storage and Stability

On receipt entire kit should be stored at 4°C, protected from light. Upon opening, use kit within 6 months. Avoid freeze-thaw cycles.

Materials Supplied

Item	Quantity	Storage Condition
Pre-Coated Microtiter Plate	6 x 16 well strips	4°C
Wash Buffer (10X)	2 x 30 mL	4°C
Diluent (10X)	2 x 30 mL	4°C
100X Detection Antibody	60 µL	4°C
Detector (100X)	150 µL	4°C
Human Nampt Standard	1 vial	4°C
TMB	12 mL	4°C
Stop Solution	12mL	4°C
Plate Sealers	2 units	4°C
Lysis Buffer (10X)	12 mL	4°C

Reagent Preparation

- Reagents must be stored at 2 - 8°C when not in use.
- Bring reagents to room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

Wash Buffer: Dilute the concentrated washing buffer (20X) with distilled water.

Day 1 (We recommend the Samples and Standards be run in duplicate)

Standard Preparation

Reconstitute Human Nampt Standard with 1 ml of dH2O to produce a stock solution (32 ng/ml). Mix the Stock solution to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The reconstituted standard should be aliquoted and stored at -20°C.

Sample Preparation

Cell lysates:
Ice-cold 1X Lysis Buffer and 1X Diluent are prepared by 1:9 dilutions with dH2O and placed on ice until needed. Grow cells to 80-90 % confluency. Adherent cells can be scraped off plate and transferred to a tube; suspension cells pipetted to appropriate tube. Centrifuge at 700-1000 x g for 5 min at 4°C and carefully remove and discard supernatant. Wash 1-2 times with ice-cold PBS. Add 200 µl ice-cold 1X Lysis Buffer with 1 mM PMSF (not inluded) per 1 x 10^7 cells and allow to stand on ice for 30 min. Centrifuge at 10K x g for 5 min at 4°C and transfer supernatant to a new tube. The supernatant is the cell lysate and should be freshly prepared and diluted into 1X Diluent. As a starting point 1/10 to 1/1000 dilutions are recommended. If samples fall outside the assay range a lower or higher dilution may be required.

Standard Curve preparation

Prepare Standard Curve using 2-fold serial dilutions with 1X Diluent

Dilution number	Nampt Standard	1X Diluent	Final concentration (ng/ml)
1	300 µL (32 ng/ml)	300 µL	16
2	300 µL (16 ng/ml)	300 µL	8
3	300 µL (8 ng/ml)	300 µL	4
4	300 µL (4 ng/ml)	300 µL	2
5	300 µL (2 ng/ml)	300 µL	1
6	300 µL (1 ng/ml)	300 µL	0.5
7	300 µL (0.5 ng/ml)	300 µL	0.25
8	-	300 µL	0

- Determine the number of 16-well strips needed for the assay and insert them into the frame for current use. The extra strips should be resealed in the foil pouch and can be stored at 4°C for up to 1 month.
- Add 100 µl of the Standards and Samples into the appropriate wells in duplicate.
- Cover the plate with plate sealer and incubate at 4°C overnight

Day 2: (Note: the Detector must be used within 1 hr of preparation)
Assay Protocol

1. Prepare 1X Wash Buffer: Dilute 10X Wash Buffer 1:9 with dH2O.
2. Warm Detection Antibody to room temperature. Dilute the antibody 1:250 in 1X Diluent (8 µl antibody + 1992 µl 1X Diluent). Diluted antibody cannot be stored.
3. Remove plate from 4°C, aspirate and wash 3 times with 300 µl of 1X Wash Buffer.
4. After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.
5. Add 100 µl of Detection Antibody to each well.
6. Cover plate with plate sealer and incubate for 1 hr at 37°C.
7. After about 30-45 min prepare 1X Detector: Dilute 100X Detector 1:99 with 1X Diluent (100 µl Detector + 9.9 ml of 1X Diluent).
8. Remove plate from 37°C, aspirate and wash 3 times with 300 µl of 1X Wash Buffer.
9. After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.
10. Add 100 µl of 1X Detector to each well.
11. Cover plate with plate sealer and incubate for 1 hr at 37°C.
12. Warm the TMB Solution and Stop Solution to room temperature.
13. Remove plate from 37°C, aspirate and wash 5 times with 300 µl of 1X Wash Buffer.
14. After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.
15. Add 100 µl of TMB Solution to each well.
16. Allow the color to develop at room temperature in the dark for 10 min.
17. Stop the reaction by adding 100 µl of Stop Solution to each well.
18. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue product that turns yellow when Stop Solution is added.
    Caution: *Stop Solution is Corrosive*
19. Measure the OD at 450 nm in an ELISA plate reader within 30 min.

Calculation:

1. Average the duplicate readings for each standard and Test Sample.
2. Subtract the average 0 ng/ml standard from each of the above.
3. Generate a Standard Curve by plotting the average absorbances on the horizontal (X) axis vs. the corresponding concentration (ng/ml) on the vertical (Y) axis.
4. Calculate the Test Sample Nampt concentrations by interpolation of the Standard Curve regression curve as shown above in the form of a quadratic equation.
5. If the Test Samples were diluted, multiply the interpolated values by the dilution
6. factor to calculate the corrected human Nampt serum concentrations.

Assay Precision

Intra-assay and Inter-assay Precision: (2) samples of known concentration were assayed in replicates (10) times to test precision within an assay.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	2.73 - 9.76	4.02 - 7.40

Technical Hints and Limitations:

- It is recommended that all standards and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the
- plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock
- solutions. Crystals are readily dissolved at room temperature or at 37°C before
- dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at
- any time during the assay.
- Keep TMB Substrate Solution protected from light.
- The Stop Solution consists of Sulfuric acid. Although diluted, the Stop Solution
- should be handled with gloves, eye protection and protective clothing.

Technical Support

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