DATA SHEET

Nampt (Visfatin/PBEF) (human) Intracellular ELISA Kit

(Catalog #K4909-100; 100 assays; Store kit at 4°C)

I. Description:

Nampt, nicotinamide phosphoribosyltransferase, is the rate limiting enzyme of the mammalian NAD biosynthesis pathway. The circulating NMN (nicotinamide mononucleotide) and NAD are taken up by beta cells and converted to NAD by Nmnat (nicotinamide mononucleotide adenyltransferase) and intracellular Nampt. The Nampt plays a critical role in enhancing life span and protecting against oxidative damage. The Nampt (Visfatin/PBEF) (human) Intracelluar ELISA Kit is to be used for the *in vitro* quantitative determination of intracellular mouse or rat Nampt (Visfatin/PBEF). This assay is a sandwich ELISA which utilizies a 96-well microtiter plate which was pre-coated with a monoclonal antibody and a purified polyclonal detection antibody. A HRP-conjugated anti-IgG and TMB (3,3',5,5'-tetramethylbenzidine) is added to generate a color intensity directly proportional to the concentration of Nampt in the samples. This ELISA is specific for the measurement of natural and recombinant human Nampt. It does not cross-react with human adiponectin, human resistin, human RELM-β, human leptin, human GPX3, human ANGPTL4, human FABP4, human ANGPTL6, human PAI1. The assay range is 0.063 – 16 ng Nampt/ml and a detection limit of 30 pg/ml (based on adding two standard deviations to the mean value of the zero standard).

II. Kit Contents:

Component	100 Assays	Part Number
Pre-coated Microtiter Plate	1 ea (12 x 8 well strips)	K4909-100-1
Wash Buffer (10X)	50 ml	K4909-100-2
Diluent (5X)	50 ml	K4909-100-3
Detection Antibody	12 ml	K4909-100-4
Detector 100X (Hrp conjugated anti-IgG)	150 µl	K4909-100-5
Human Nampt Standard (lyophilized,32 ng)	1 vial	K4909-100-6
Human Nampt QC Sample (lyophilized)	1 vial	K4909-100-7
TMB Substrate Solution	12 ml	K4909-100-8
Stop Solution	12 ml	K4909-100-9
Plate Sealers	3 each	K4909-100-10
Lysis Buffer (10X)	12 ml	K4909-100-11

III. Storage Conditions:

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.

IV. Assay Procedure (Read the ENTIRE Protocol Before Proceeding)

1. Day 1: (We recommend the Samples, Standards and QC Sample be run in duplicate)

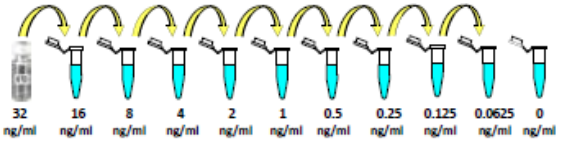
a) **Cells Lysates**: Ice-cold 1X Lysis Buffer and 1X Diluent are prepared by 1:9 and 1:4 dilutions with dH2O, respectively and placed on ice until needed. Grow cells to 80-90 % confluency. Adherent cells can be scraped off plate and transferred to a tube; suspension cells pipetted to appropriate tube. 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.

b) **QC Sample**: Reconstitute Human Nampt QC Sample with 1 ml of dH_2O. Mix the QC Sample to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The QC Sample is ready to use-do not dilute it (refer to the C of A for current QC Sample concentration).

c) **Standards**: Reconstitute Human Nampt Standard with 1 ml of dH_2O 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.

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

To obtain	Add	Into
16 ng/ml	300 µl of Nampt (32 ng/ml)	300 µl of 1X Diluent
8 ng/ml	300 µl of Nampt (16 ng/ml)	300 µl of 1X Diluent
4 ng/ml	300 µl of Nampt (8 ng/ml)	300 µl of 1X Diluent
2 ng/ml	300 µl of Nampt (4 ng/ml)	300 µl of 1X Diluent
1 ng/ml	300 µl of Nampt (2 ng/ml)	300 µl of 1X Diluent
0.5 ng/ml	300 µl of Nampt (1 ng/ml)	300 µl of 1X Diluent
0.25 ng/ml	300 µl of Nampt (0.5 ng/ml)	300 µl of 1X Diluent
0.125 ng/ml	300 µl of Nampt (0.25 ng/ml)	300 µl of 1X Diluent
0.0625 ng/ml	300 µl of Nampt (0.125 ng/ml)	300 µl of 1X Diluent
0 ng/ml	300 µl of 1X Diluent	Empty tube



e) Determine the number of 8-well strips needed for 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.

f) Add 100 µl of the Standards, Samples and QC Sample into the appropriate wells in duplicate.

g) Cover the plate with plate sealer and incubate at 4°C overnight.

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

a) Prepare 1X Wash Buffer: Dilute 10X Wash Buffer 1:9 with dH_2O.

b) Warm Detection Antibody to room temperature.

c) Remove plate from 4°C, aspirate and wash x 3 with 300 µl of 1X Wash Buffer.

d) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.

e) Add 100 µl of Detection Antibody to each well.

f) Cover plate with plate sealer and incubate for 1 hr at 37°C.

g) After about 30-45 min prepare 1X Detector: Dilute 100X Detector 1:99 with 1X Diluent.

h) Remove plate from 37°C, aspirate and wash x 3 with 300 µl of 1X Wash Buffer.

i) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.

j) Add 100 µl of 1X Detector to each well.

k) Cover plate with plate sealer and incubate for 1 hr at 37°C.

l) Warm the TMB Substrate Solution and Stop Solution to room temperature.

m) Remove plate from 37°C, aspirate and wash x 5 with 300 µl of 1X Wash Buffer.

n) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.

o) Add 100 µl of TMB Substrate Solution to each well.

p) Allow the color to develop at room temperature in the dark for 10 min.

q) Stop the reaction by adding 100 µl of Stop Solution to each well.

r) Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
Caution: Stop Solution is a Corrosive Solution

s) Measure the OD at 450 nm in an ELISA plate reader within 30 min.

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