DATA SHEET

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

(Catalog #K4908-100; 100 assays; Store kit at 4°C)

I. Description:

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, a secretory form of Nampt (nicotinamide phosphoribosy-ltransferase), corresponds to pre-B cell colony-enhancing factor (PBEF), a 52 kDa cytokine expressed in lymphocytes. 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. The Nampt (Visfatin/PBEF) (mouse/rat) Serum ELISA Kit is to be used for the *in vitro* quantitative determination of mouse or rat Nampt (Visfatin/PBEF) in serum. 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 mouse and rat Nampt. It does not cross-react with human Nampt, human adiponectin, mouse adiponectin, mouse resistin, mouse vaspin, mouse RBP4, mouse GPX3, mouse progranulin, mouse IL-33, mouse clusterin, mouse ANGPTL3, mouse ANGPTL4, mouse ANGPTL6, mouse leptin, mouse TNF-α. The assay range is 0.5 – 32 ng Nampt/ml and a detection limit of 50 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)	K4908-100-1
Wash Buffer (10X)	50 ml	K4908-100-2
Diluent (5X)	50 ml	K4908-100-3
Detection Antibody	12 ml	K4908-100-4
Detector 100X (Hrp conjugated anti-IgG)	150 µl	K4908-100-5
Mouse Nampt Standard (lyophilized,64 ng)	1 vial	K4908-100-6
Mouse Nampt QC Sample (lyophilized)	1 vial	K4908-100-7
TMB Substrate Solution	12 ml	K4908-100-8
Stop Solution	12 ml	K4908-100-9
Plate Sealers	3 each	K4908-100-10

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.

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

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

a) **Serum**: Use a serum separator tube. Let samples clot at room temperature for 30 min before centrifugation for 20 min at 1000 x g. Assay freshly prepared serum or store serum in aliquots at -20 C for future use. Avoid repeated freeze/thaw cycles. Serum should be diluted in Diluent 1X. Samples containing visible precipitates must be clarified before use. If samples fall outside the assay range a lower or higher dilution may be required.Allow all samples and kit components to equilibrate to room temperature (20 - 25°C).

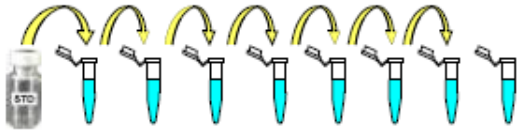
b) **QC Sample**: Reconstitute Mouse Nampt QC Sample with 1 ml of dH_2O. Mix the QC Sample to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The QC Sample is ready to use-do not dilute it (refer to the C of A for current QC Sample concentration).

c) **Standards**: Reconstitute Mouse Nampt Standard with 1 ml of dH_2O to produce a stock solution (64 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 1X Diluent: Dilute 5X Diluent 1:4 with dH_2O.

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

To obtain	Add	Into
32 ng/ml	300 µl of Nampt (64 ng/ml)	300 µl of 1X Diluent
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 ng/ml	300 µl of 1X Diluent	Empty tube



f) 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.

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

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

2. Day 2:

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.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium