

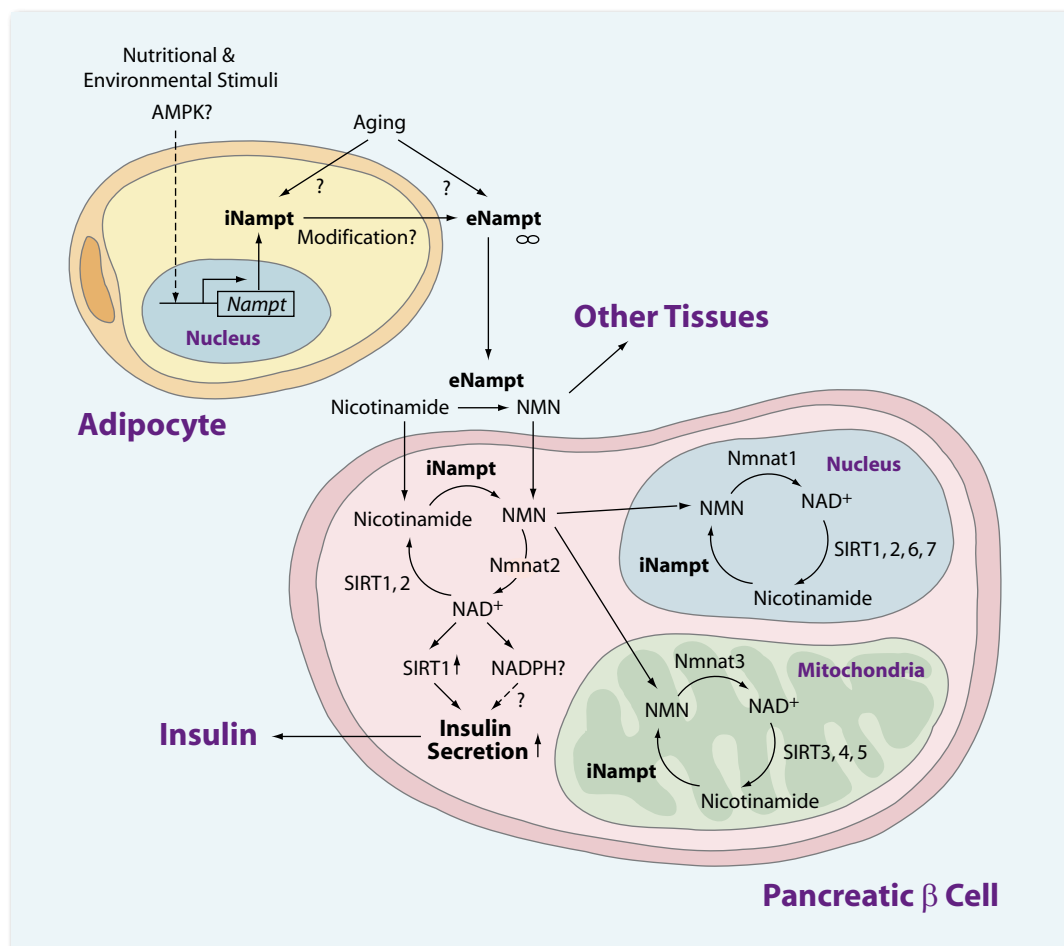
## NAMPT [PBEF/Visfatin]

### Introduction

The NAD<sup>+</sup> biosynthesis pathway in mammals consists of two steps involving the enzymes nicotinamide phosphoribosyltransferase (NAMPT [EC 2.4.2.12.]) and nicotinamide/nicotinic acid mononucleotide adenylyltransferase (Nmnat). NAMPT converts nicotinamide into nicotinamide mononucleotide (NMN) which then is converted to NAD<sup>+</sup> by Nmnat. The rate limiting enzyme in the

biosynthetic pathway of NAD<sup>+</sup> is NAMPT [1]. Originally, NAMPT was identified as pre-B cell colony enhancing factor (PBEF) a presumptive cytokine-like protein involved in subclinical inflammation [2, 3]. Later the protein was renamed visfatin, a visceral fat-derived adipocytokine believed to mimic

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**FIGURE:** NAD<sup>+</sup> biosynthesis in pancreatic β cells in each subcellular compartment.

Adapted from: *Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme*: J.R. Revollo, et al.; Cell Metab. 6, 363 (2007) and *Nicotinamide phosphoribosyltransferase (Nampt): a link between NAD biology, metabolism, and diseases*: S. Imai; Curr. Pharm. Des. 15, 20 (2009)



insulin action [4]. However this report was controversial resulting in the retraction of the paper [5]. Although NAMPT may not directly bind to the insulin receptor as originally thought, NAMPT does regulate insulin secretion in  $\beta$  cells [6] and can function as a cell survival factor [7].

NAMPT forms dimers [8] and has been shown to be secreted. Secretion of NAMPT is not inhibited by brefeldin A, suggesting a non-classical secretory pathway for NAMPT [6]. Two forms of NAMPT exist, an intracellular form (iNAMPT) and an extracellular form (eNAMPT). While the function of iNAMPT as an essential and rate-limiting NAD<sup>+</sup> biosynthetic enzyme is well established, the physiological role of eNAMPT is still a matter of debate [9]. It has been suggested that eNAMPT converts nicotinamide into extracellular NMN in the blood which functions as a systemic signaling molecule regulating  $\beta$  cell function [6, 10]. Recently, eNAMPT has also been shown to activate pro-inflammatory signaling and to be involved in the induction of inducible nitric oxide synthase (iNOS; iNOS II) through an ERK1/2 NF- $\kappa$ B-dependent mechanism [11].

NAD<sup>+</sup> is a classic coenzyme in cellular redox reactions and hence is involved in a broad range of biological functions. Of particular interest is its regulatory role of the NAD-dependent deacetylase sirtuin 1 (SIRT1; Sirt1; Sir2 (yeast) activity [1]. SIRT1 is a deacetylase that requires NAD<sup>+</sup> for its function [1, 12]. By modifying chromatin-associated proteins SIRT1 plays an important role in the epigenetic regulation of metabolism in response to nutrient availability [13]. NAMPT and SIRT1 together with a third enzyme AMP-activated protein kinase (AMPK) function as a cellular signaling checkpoint sensing and reacting to nutrient availability [14].

NAMPT-mediated NAD<sup>+</sup> biosynthesis has widespread actions on cellular senescence, aging, obesity, diabetes, cancer, and inflammation.

Interestingly, NAMPT expression levels show circadian oscillation. A circadian clock feedback cycle exists where clock genes together with SIRT1 regulate NAMPT expression [15, 16]. Thus, SIRT1 controls the synthesis of NAD<sup>+</sup>, its own cofactor.

A systemic regulatory network for mammalian aging, called "NADWorld" has been proposed [17, 18]. NAD<sup>+</sup> biosynthesis and the dependence of SIRT1 on NAD<sup>+</sup> are central to this hypothesis. Cells such as pancreatic  $\beta$  cells and neurons that do not have adequate amounts of iNAMPT would depend on systemic NMN biosynthesis by eNAMPT for NAD<sup>+</sup>. If systemic NAD<sup>+</sup> biosynthesis declines, these cells would exhibit functional problems due to inadequate NAD<sup>+</sup> availability and the corresponding reduction in SIRT1 activity. This situation might contribute to typical age-associated complications such as type 2 diabetes [19].

#### LITERATURE REFERENCES:

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#### SELECTED REVIEW ARTICLES

- The regulation of nicotinamide adenine dinucleotide biosynthesis by Nampt/PBEF/visfatin in mammals: J. R. Revollo, et al.; *Curr. Opin. Gastroenterol.* **23**, 164 (2007)
- Energy-responsive timekeeping: D. A. Bechtold; *J. Genet.* **87**, 447 (2008)
- Nicotinamide phosphoribosyltransferase (Nampt): a link between NAD biology, metabolism, and diseases: S. Imai; *Curr. Pharm. Des.* **15**, 20 (2009)
- Therapeutic potential of SIRT1 and NAMPT-mediated NAD biosynthesis in type 2 diabetes: S. Imai & W. Kiess; *Front. Biosci.* **14**, 2983 (2009)
- The NAD World: a new systemic regulatory network for metabolism and aging--Sirt1, systemic NAD biosynthesis, and their importance: S. Imai; *Cell Biochem. Biophys.* **53**, 65 (2009)
- From heterochromatin islands to the NAD World: A hierarchical view of aging through the functions of mammalian Sirt1 and systemic NAD biosynthesis: S. Imai; *Biochim. Biophys. Acta* **1790**, 997 (2009)
- Nampt: linking NAD biology, metabolism and cancer: A. Garten, et al.; *Trends Endocrinol. Metab.* **20**, 130 (2009)

## Proteins

Product	Source/Host	Prod. No.	Size
<b>Nampt (human) (rec.)</b>	Produced in HEK 293 cells. Full length human Nampt (visfatin/PBEF) (aa 1-491) is fused at the N-terminus to a FLAG <sup>®</sup> -tag.	AG-40A-0031-C010 AG-40A-0031-C050	10 $\mu$ g 50 $\mu$ g
<b>Nampt (human) (rec.) (His)</b>	Produced in <i>E. coli</i> . Full length human Nampt (visfatin/PBEF) (aa 1-491) is fused at the C-terminus to a His-tag.	AG-40A-0018-C050	50 $\mu$ g
<b>Nampt (mouse) (rec.)</b>	Produced in HEK 293 cells. Full length mouse Nampt (visfatin/PBEF) (aa 1-491) is fused at the N-terminus to a FLAG <sup>®</sup> -tag.	AG-40A-0056-C010 AG-40A-0056-C050	10 $\mu$ g 50 $\mu$ g
<b>Nampt (mouse) (rec.) (His)</b>	Produced in <i>E. coli</i> . Full length mouse Nampt (visfatin/PBEF) (aa 1-491) is fused at the C-terminus to a His-tag.	AG-40A-0017-C050	50 $\mu$ g
<b>Nampt (rat) (rec.)</b>	Produced in HEK 293 cells. Full length rat Nampt (Visfatin/PBEF) (aa 1-491) is fused at the N-terminus to a FLAG <sup>®</sup> -tag.	AG-40A-0058-C010 AG-40A-0058-C050	10 $\mu$ g 50 $\mu$ g
<b>Nampt (rat) (rec.) (His)</b>	Produced in <i>E. coli</i> . Full length rat Nampt (visfatin/PBEF) (aa 1-491) is fused at the C-terminus to a His-tag.	AG-40A-0027-C050	50 $\mu$ g

## Antibodies

Product	Specificity	Application	Prod. No.	Size
<b>anti-Nampt, mAb (OMNI379)</b> Isotype: Mouse IgG2	Recognizes human, mouse and rat Nampt.	IHC (PS), ICC, IP, WB	AG-20A-0034-C050	50 µg
			AG-20A-0034-C100	100 µg
			AG-20A-0034B-C050 Biotin	50 µg
<b>anti-Nampt (human), pAb</b>	Recognizes human Nampt. Weakly cross-reacts with mouse Nampt.	WB	AG-25A-0025-C100	100 µg
			AG-25A-0025B-C050 Biotin	50 µg
<b>anti-Nampt (human), pAb (CT)</b>	Recognizes human Nampt. Weakly cross-reacts with mouse Nampt.	IHC (PS), WB	AG-25A-0027-C100	100 µg
<b>anti-Nampt (human), pAb (NT)</b>	Recognizes human Nampt. Weakly cross-reacts with mouse Nampt.	WB	AG-25A-0026-C100	100 µg
<b>anti-Nampt (mouse), pAb</b>	Recognizes mouse Nampt. Weakly cross-reacts with human Nampt.	WB	AG-25A-0028-C100	100 µg
<b>anti-Nampt (rat), pAb</b>	Recognizes human (weak) and rat Nampt.	WB	AG-25A-0033-C100	100 µg

## Nampt ELISA Kits

### Nampt (human) ELISA Kit

AG-45A-0006EK-KI01		1 x 96 wells
AG-45A-0006TP-KI01	Twin Plex	2 x 96 wells
AG-45A-0006PP-KI01	Penta Plex	5 x 96 wells

For the quantitative determination of human Nampt (visfatin/PBEF) in serum. **SENSITIVITY:** 30pg/ml (range 0 to 16ng/ml).

### The Standard! – Cited in many publications!

#### PRODUCT SPECIFIC LITERATURE REFERENCES:

- Visfatin is present in bovine mammary epithelial cells, lactating mammary gland and milk, and its expression is regulated by cAMP pathway: T. Yonezawa, et al.; *FEBS Lett.* **580**, 6635 (2006)
- Molecular characteristics of serum visfatin and differential detection by immunoassays: A. Körner, et al.; *J. Clin. Endocrinol. Metab.* **92**, 4783 (2007)
- Serum levels of the adipokine visfatin are increased in pre-eclampsia: M. Fasshauer, et al.; *Clin. Endocrinol.* **69**, 69 (2008)
- Correlation of circulating full-length visfatin (PBEF/Nampt) with metabolic parameters in subjects with and without diabetes: a cross-sectional study: R. Retnakaran, et al.; *Clin. Endocrinol.* **69**, 885 (2008)
- Longitudinal changes in pancreatic and adipocyte hormones following Roux-en-Y gastric bypass surgery: M.M. Swarbrick, et al.; *Diabetologia* **51**, 1901 (2008)
- Adipokines influencing metabolic and cardiovascular disease are differentially regulated in maintenance hemodialysis: M. Ziegelmeier, et al.; *Metabolism* **57**, 1414 (2008)
- Ethnic-specific Correlations of Visfatin With Circulating Markers of Endothelial Inflammation and Function: M. Reimann, et al.; *Obesity Epub ahead of print*, (2009)
- Visfatin activates eNOS via Akt and MAP kinases and improves endothelial cell function and angiogenesis in vitro and in vivo: translational implications for atherosclerosis: F. Lovren, et al.; *Am. J. Physiol. Endocrinol. Metab.* **296**, E1440 (2009)
- Circulating visfatin in chronic obstructive pulmonary disease: X. Liu, et al.; *Nutrition* **25**, 373 (2009)
- Visfatin in gestational diabetes: serum level and mRNA expression in fat and placental tissue: B. Telejko, et al.; *Diabetes Res. Clin. Pract.* **84**, 68 (2009)
- Plasma visfatin concentrations after a lifestyle intervention were directly associated with inflammatory markers: S. Bo, et al.; *Nutr. Metab. Cardiovasc. Dis.* **19**, 423 (2009)

### new Nampt (human) (IntraCellular) ELISA Kit

AG-45A-0008EK-KI01		1 x 96 wells
AG-45A-0008TP-KI01	Twin Plex	2 x 96 wells

For the quantitative determination of Nampt (visfatin/PBEF) in human cell lysates. **SENSITIVITY:** 30pg/ml (range 0 to 32ng/ml).

### Nampt (mouse/rat) Dual ELISA Kit

AG-45A-0007EK-KI01		1 x 96 wells
AG-45A-0007TP-KI01	Twin Plex	2 x 96 wells
AG-45A-0007PP-KI01	Penta Plex	5 x 96 wells

For the quantitative determination of Nampt (Visfatin/PBEF) in mouse or rat serum. **SENSITIVITY:** 50pg/ml (range 0 to 64ng/ml).

### new Nampt (mouse/rat) (IntraCellular) Dual ELISA Kit

AG-45A-0009EK-KI01		1 x 96 wells
AG-45A-0009ETP-KI01	Twin Plex	2 x 96 wells

For the quantitative determination of Nampt (Visfatin/PBEF) in mouse or rat cell lysates. **SENSITIVITY:** 50pg/ml (range 0 to 64ng/ml).

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# Sirtuins

## Proteins

### Sirtuin 1 (human) (rec.) (His)

AG-40A-0117-C010 10 µg  
AG-40A-0117-C050 50 µg

Produced in *E. coli*. The mature peptide of human sirtuin 1 (aa 2-555) is fused at the N-terminus to a His-tag.

### Sirtuin 2 (human) (rec.) (His)

AG-40A-0121-C010 10 µg  
AG-40A-0121-C050 50 µg

Produced in *E. coli*. The mature peptide of human sirtuin 2 (aa 2-389) is fused at the N-terminus to a His-tag.

### new Sirtuin 5 (Intact Form) (human) (rec.) (His)

AG-40A-0139-C010 10 µg  
AG-40A-0139-C050 50 µg

Produced in *E. coli*. The full-length peptide of human sirtuin 5 (aa 1-310) is fused at the N-terminus to a His-tag.

### new Sirtuin 6 (human) (rec.) (His)

AG-40A-0140-C010 10 µg  
AG-40A-0140-C050 50 µg

Produced in *E. coli*. The mature peptide of human sirtuin 6 (aa 1-355) is fused at the N-terminus to a His-tag.

## Antibodies

### new anti-Sirtuin-1 (human), mAb (SR119-1AG)

AG-20A-0081-C050 50 µg  
AG-20A-0081-C100 100 µg

CLONE: SR119-1AG. ISOTYPE: Mouse IgG1. IMMUNOGEN: Recombinant human sirtuin 1. SPECIFICITY: Recognizes human sirtuin 1. APPLICATION: WB.

### anti-Sirtuin 1 (human), pAb

AG-25A-0082-C100 100 µg

From rabbit. IMMUNOGEN: Recombinant human sirtuin 1. SPECIFICITY: Recognizes human sirtuin 1. APPLICATION: WB.

### new anti-Sirtuin 2 (human), mAb (S2R233-1)

AG-20A-0076-C050 50 µg  
AG-20A-0076-C100 100 µg

CLONE: S2R233-1. ISOTYPE: Mouse IgG2. IMMUNOGEN: Recombinant human sirtuin 2. SPECIFICITY: Recognizes human sirtuin 2. APPLICATION: WB.

### anti-Sirtuin 2 (human), pAb

AG-25A-0083-C100 100 µg

From rabbit. IMMUNOGEN: Recombinant human sirtuin 2. SPECIFICITY: Recognizes human sirtuin 2. APPLICATION: WB.

### new anti-Sirtuin 5 (human), pAb

AG-25A-0100-C100 100 µg

From rabbit. IMMUNOGEN: Recombinant human sirtuin 5. SPECIFICITY: Recognizes human sirtuin 5. APPLICATION: WB.

### new anti-Sirtuin 6 (human), pAb

AG-25A-0101-C100 100 µg

From rabbit. IMMUNOGEN: Recombinant human sirtuin 6. SPECIFICITY: Recognizes human sirtuin 6. APPLICATION: WB.

## LATEST INSIGHT

### NAMPT is Essential for G-CSF-induced Myeloid Differentiation

J. Skokowa, et al. identified NAMPT as an essential enzyme mediating granulocyte colony-stimulation factor (G-CSF)-triggered granulopoiesis in healthy individuals and in individuals with severe congenital neutropenia. The molecular events induced by NAMPT include NAD<sup>+</sup>-dependent activation of sirtuin 1 (SIRT1), induction of CCAAT/enhancer binding proteins  $\alpha$  and  $\beta$ , and upregulation of G-CSF synthesis and G-CSF receptor expression. Their results point out a role of the NAD<sup>+</sup> metabolic pathway in G-CSF-triggered myelopoiesis.

**LIT:** NAMPT is essential for the G-CSF-induced myeloid differentiation via a NAD(+)-sirtuin-1-dependent pathway: J. Skokowa, et al.; Nat. Med. 15, 151 (2009)

## LATEST INSIGHT

### A Rare NAMPT Gene Variation is Associated with Protection from Obesity

A.I. Blakemore, et al. identified a rare variant in the NAMPT gene that is associated with protection from obesity. One single-nucleotide polymorphism (SNP), rs1048781, located in intron 4 of the NAMPT gene was linked with severe obesity.

**LIT:** A rare variant in the visfatin gene (NAMPT/PBEF1) is associated with protection from obesity: A.I. Blakemore, et al.; Obesity 17, 1549 (2009)

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