Human NAMPT Rabbit Polyclonal Antibody

Cat# CY-P1038

100 µg (1.0 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB, E	**H**	**50-55 kDa**	**Rabbit IgG**

Background

Nicotinamide phosphoribosyltransferase (NAMPT), also known as pre-B-cell colony-enhancing factor, is the rate-limiting enzyme that converts nicotinamide to nicotinamide mononucleotide (NMN) from nicotinamide in the salvage pathway of NAD+ biosynthesis in mammals. Nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1) converts NMN to NAD+. The expression of NAMPT is upregulated during activation of immune cells such as monocytes, macrophages, dendritic cells, T and B cells, as well as in amniotic epithelial cells upon stimulation with several inflammatory cytokines. NAMPT-specific inhibitor, FK866 was found to deplete intracellular NAD content, resulting in apoptotic cell death in many cancer cell lines without any DNA damaging effect. Recently, Nakahata K et al, demonstrated that NAMPT is required to modulate circadian gene expression and circadian oscillation of NAD+.

Specificity/Sensitivity: Human NAMPT Polyclonal Antibody detects endogenous NAMPT by western blotting, but not immunoprecipitation.

Source/Purification: This polyclonal antibody is produced by immunizing rabbit with a recombinant NAMPT, corresponding full length of human NAMPT, expressed in *E. coli*. IgG is purified by antigen-affinity chromatography.

Recommended Antibody Dilutions: Western blotting: 1-2 µg/ml.

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western **IP:** Immunoprecipitation **IHC:** Immunohistochemistry **IC:** Immunocytochemistry **F:** Flow cytometry **E:** ELISA **FP:** Fluorescence Polarization assay
Species Cross-Reactivity Key: H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Mi:** mink **C:** chicken **X:** *Xenopus* **Z:** zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

General References:

1. Revollo, J. R, Grimm, A. A, Imai, S. (2004) J. Biol. Chem. 279: 50764-50763, 2004.

2. Rongvaux A, Shea RJ, Mulks MH, Gigot D, Urbain J, et al. (2002) Eur J Immunol 32: 3225–3234.

3. Iqbal J, Zaidi M (2006) Biochem Biophys Res Commun 342: 1312–1318.

4. Nau GJ, Richmond JF, Schlesinger A, Jennings EG, Lander ES, et al. (2002) Proc Natl Acad Sci 99: 1503–1508.

5. Huang Q, Liu D, Majewski P, Schulte LC, Korn JM, et al. (2001) Science 294: 870–875.

6. Ognjanovic S, Bao S, Yamamoto SY, Garibay-Tupas J, Samal B, et al. (2001) J Mol Endocrinol 26: 107–117.7. Max Hasmann and Isabel Schemainda (2003) Cancer Res. 63: 7436 - 7442.

7. M. Hasmann and I. Schemainda (2003) Cancer Res. 63, 7436-7442.

8. Kathryn Moynihan Ramsey, et al. (2009) Science 324, 651

9. Yasukazu Nakahata, et al. (2009) Science 324, 654

Fig.1 WB analysis of human NAMPT in cell lysates using the Human NAMPT rabbit polyclonal antibody

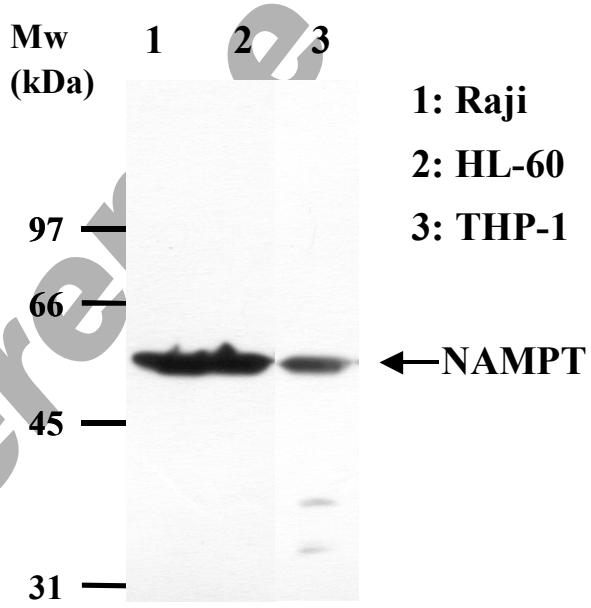
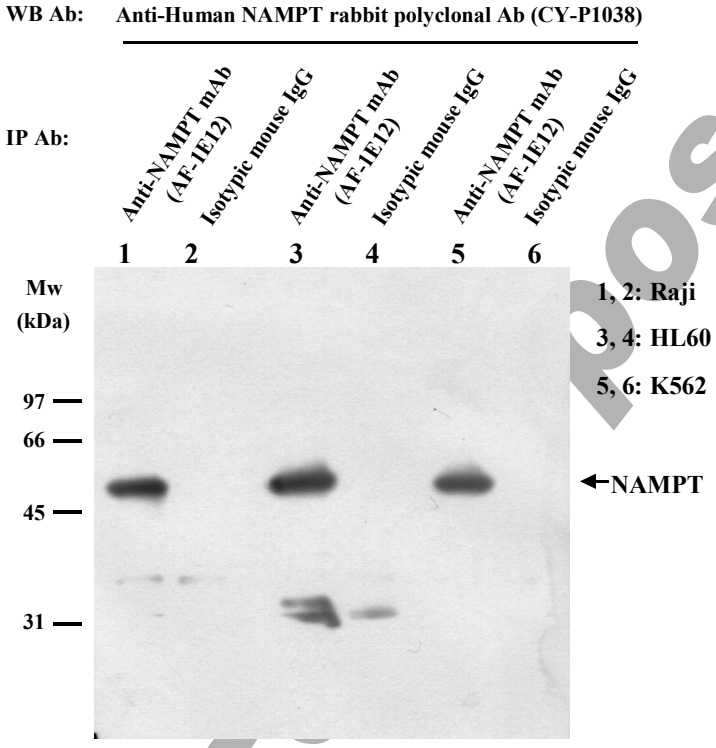


Fig.2 IP-WB analysis of human NAMPT in cell lysates of three cell lines using Human NAMPT mouse monoclonal antibody, AF-1E12 (CycLex Co., Ltd. Cat# CY-M1035) for IP and the Human NAMPT rabbit polyclonal antibody for WB.



Western blotting Protocol

Solutions and Reagents
Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3,000-5,000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

Related Products

***CycLex Human NAMPT Mouse Monoclonal Antibody (AF-1E12): Cat# CY-M1035**
***CycLex Human NAMPT Rabbit Polyclonal Antibody: Cat# CY-P1038**
***CycLex NAMPT Colorimetric Assay kit: Cat# CY-1450**
***CycLex NMNAT1 Colorimetric Assay kit: Cat# CY-1451**
***CycLex NAD+/NADH Colorimetric Assay kit: Cat# CY-1452**
***CycLex NAMPT (nicotinamide phosphoribosyltransferase): Cat# CY-E1450**
***CycLex NMNAT1 (Nicotinamide mononucleotide adenylyltransferase 1): Cat# CY-E1451**

PRODUCED BY

CycLex Co., Ltd.
1063-103 Tera-sawaoka
Ina, Nagano 396-0002
Japan
Fax: +81-265-76-7618
e-mail: info@cyclex.co.jp
URL: http://www.cyclex.co.jp