Human NAMPT Mouse Monoclonal Antibody (Clone AF-1E12)

Cat# CY-M1035

100 µg (1.0 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
AF-1E12	IP, E	H	50-55 kDa	Mouse IgG2a

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: The Human NAMPT Monoclonal Antibody (AF-1E12) detects endogenous NAMPT by immunoprecipitation, but not by western blotting.

Source/Purification: The antibody is produced by immunizing mice with a recombinant NAMPT, corresponding full length of human NAMPT, expressed in *E. coli*. IgG is purified by protein A-Sepharose chromatography.

Recommended Antibody Dilutions: Immunoprecipitation: 1-2 µg/sample.

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:

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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.

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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 Measurement of endogenous human Nampt activity in an immunoprecipitate using this human NAMPT mouse monoclonal antibody and CycLex NAMPT Colorimetric Assay kit (CycLex Co., Ltd. Cat# CY-1450)

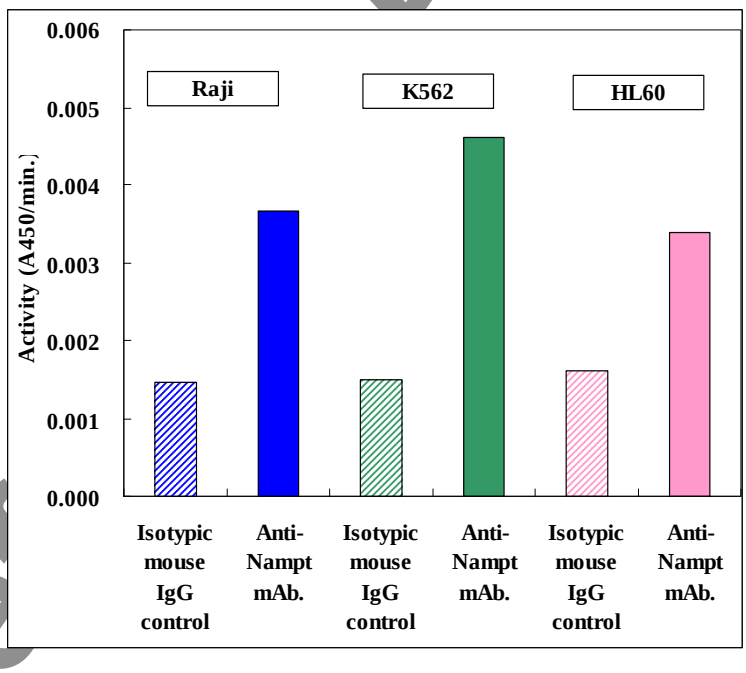
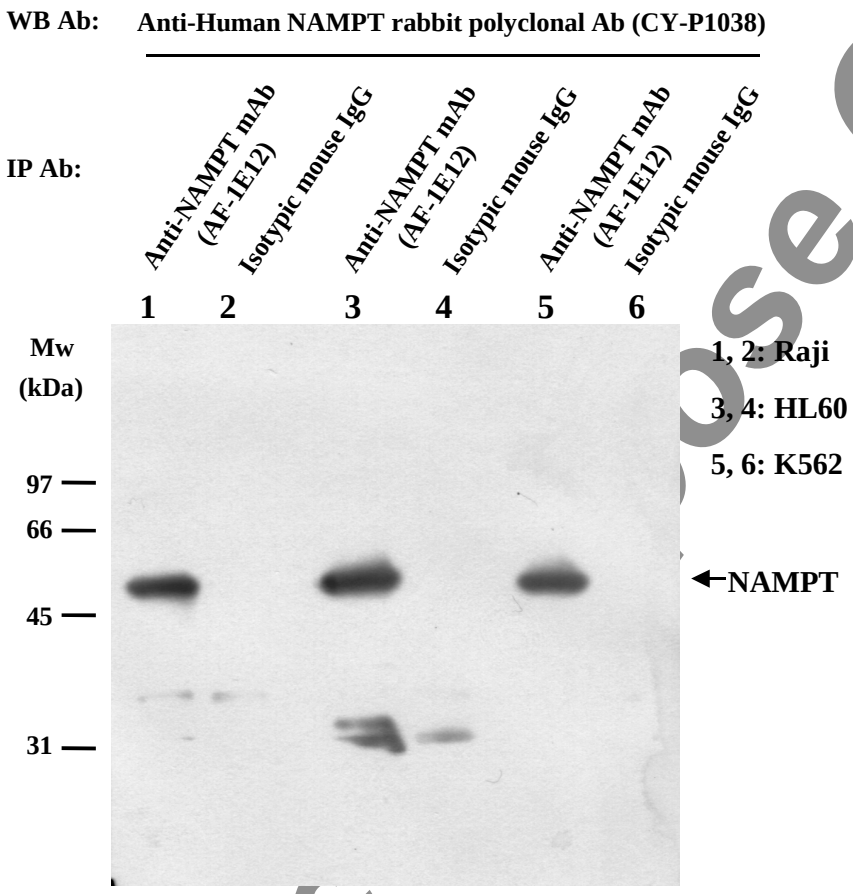


Fig.2 IP-WB analysis of human NAMPT in cell lysates of three cell lines using this human NAMPT mouse monoclonal antibody for IP and CycLex Human NAMPT rabbit polyclonal antibody (CycLex Co., Ltd. Cat# CY-P1038) for WB



Immunoprecipitation Followed by NAMPT Activity Assay Protocol

Solutions and Reagents

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

Cell Lysis Buffer (1X): 20 mM Tris (pH 8.0), 250 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Glycerolphosphate, 1 mM Na3VO4, 1 µg/ml Leupeptin
Note: We recommend adding 1 mM PMSF before use.

Protein A agarose beads: Add 5 ml of 1X PBS to 1.5 g of Protein A agarose beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)

10X TBS (Tris-buffered saline): For 1 liter of 10X TBS, use 24.2 g Tris base and 80 g NaCl. Adjust pH to 7.6 with HCl (use at 1X).

NAMPT assay buffer: 20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 20 µg/mL BSA, 12 mM $MgCl_2$

CycLex NAMPT Colorimetric Assay kit: CycLex Co., Ltd. Cat# Cat# CY-1450

Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml 1X ice-cold Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate 4 times for 5 seconds each on ice.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at –80°C.

Immunoprecipitation

1. Take 250 µl cell lysate and add Protein A Agarose Beads (40 µl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C for pre-clearance
2. Microcentrifuge for 30 seconds at 4°C. Take the supernatant and transfer to a new tube.
3. Add primary antibody and incubate with gentle rocking 4 hr to overnight at 4°C.
4. Add Protein A agarose beads (20 µl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
5. Microcentrifuge for 30 seconds at 4°C. Wash the beads 2 times with 500 µl of 1X Cell Lysis Buffer, subsequently twice with NAMPT assay buffer*. Keep on ice during washes.
6. Resuspend the beads with 20 µl NAMPT assay buffer.
7. Measure NAMPT activity by CycLex NAMPT Colorimetric Assay kit: Cat# CY-1450

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**

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