Anti-NAMPT mAb

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: Anti-NAMPT mAb (AF-1E12) detects endogenous NAMPT by immunoprecipitation, but not by western blotting.

Source/Purification: Monoclonal 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 blotting, **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 (Species enclosed in parentheses are predicted to react based on 100% sequence homology.)

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 Measurement of endogenous human Nampt activity in an immunoprecipitate using #CY-M1035 and CycLex NAMPT Colorimetric Assay kit Ver.2 (MBL Co., Ltd. CY-1251V2)

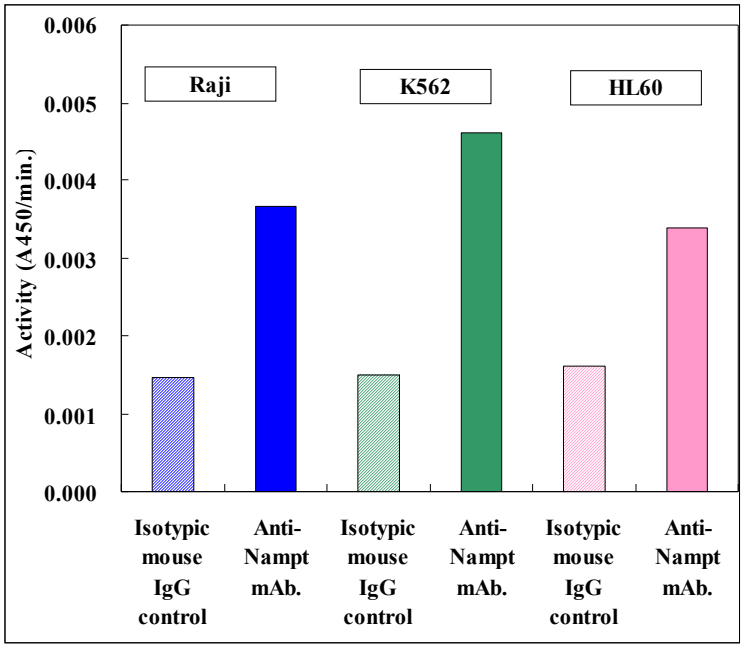
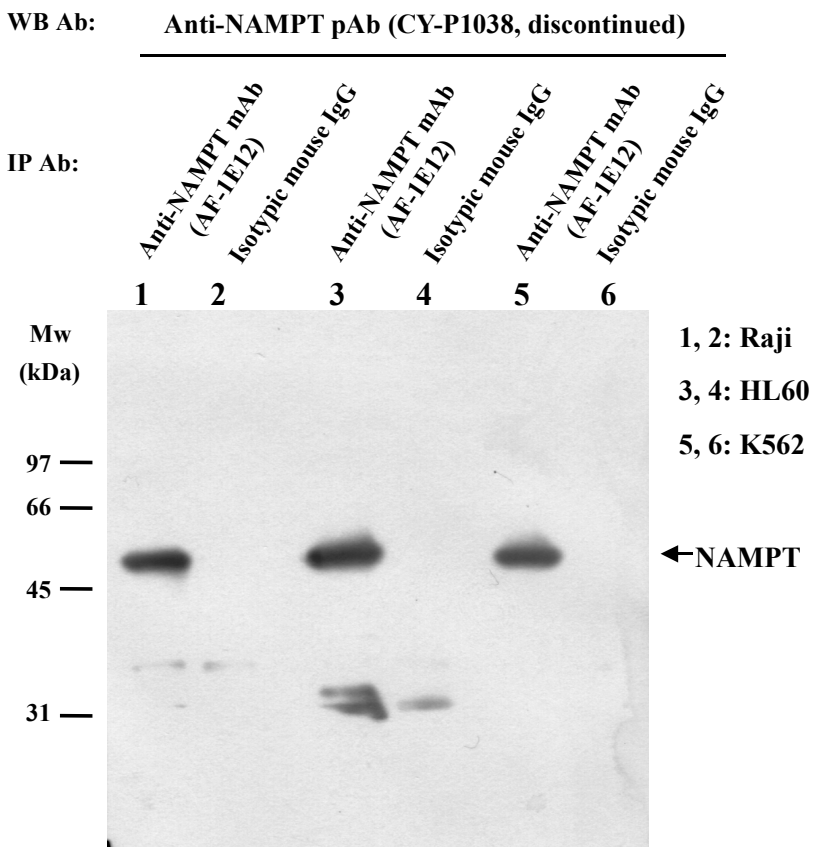


Fig.2 IP-WB analysis of human NAMPT in cell lysates of three cell lines using #CY-M1035 for IP and CycLex Anti-NAMPT pAb (MBL, #CY-P1038, discontinued) for WB



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 Na_3VO_4, 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 Ver.2: MBL Co., Ltd. Cat# CY-1251V2

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 Ver.2 (MBL Co., Ltd. CY-1251V2)

For more information, please visit our website at **https://ruo.mbl.co.jp/.**

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