Quantitative test kit for nicotinamide phosphoribosyltransferase activity

CycLex NAMPT Colorimetric Assay Kit

For 100 assays

Cat# CY-1251

Intended Use..1
Storage...1
Introduction...2
Principle of the Assay.................................2
Materials Provided.......................................3
Materials Required but not Provided...........3
Precautions...4
Detailed Protocol..5-12
Evaluation of Results...................................13
Cautions...13
Assay Characteristics...................................14
Troubleshooting...14
Reagent Stability..14
Protocol for immunoprecipitation................15
Example of Test Results...............................16-20
References..21
Related Products..22

Intended Use

The CycLex Research Product **CycLex NAMPT Colorimetric Assay Kit** detects nicotinamide phosphoribosyltransferase (NAMPT) activity in recombinant NAMPT or endogenous NAMPT immunoprecipitated from cell extract. Primarily, the CycLex Research Product **CycLex NAMPT Colorimetric Assay Kit** is designed for the rapid and sensitive evaluation of NAMPT inhibitors or activators using recombinant NAMPT. Since this kit is based on NAD+ detection system, it is not possible to directly detect NAMPT activity in crude cell extract in which NAD+ concentration is relatively high.

Applications for this kit include:
1) Screening inhibitors or activators of NAMPT.
2) Detecting the effects of pharmacological agents on NAMPT.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at -70°C.
- Don't expose reagents to excessive light.

For Reference Purpose Only!

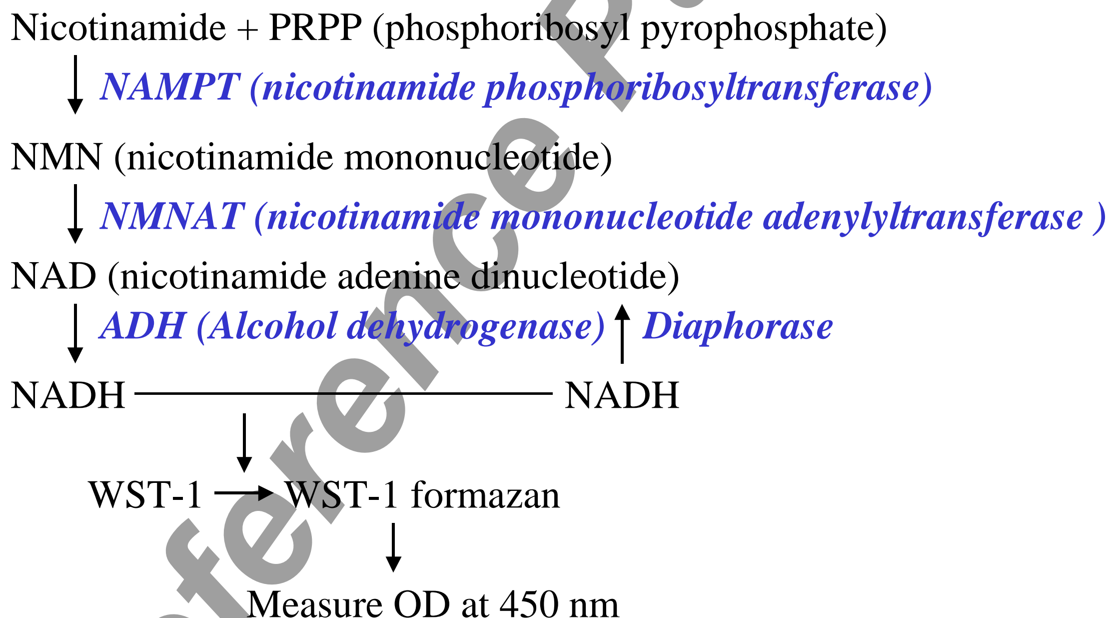
Introduction

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+.

Principle of the Assay

Since it is very simple to measure and it can be performed at a low price, the measurement of NAMPT activity in most laboratories is possible if they are equipped with a microtiter plate reader. Considering that the use of fully automatic apparatus to monitor the absorbance has become widespread, NAMPT activity measurement, which could not be made by the conventional method, is now possible with the CycLex NAMPT Colorimetric Assay Kit using the same equipment. This new method of measurement shall dramatically raise the efficiency of inhibitor screening and biochemical analysis of this enzyme.

Measuring Principle of The CycLex NAMPT Colorimetric Assay Kit



For Reference Purpose Only!

Materials Provided

Each kit contains

Materials	Quantity	Storage
① 10X NAMPT assay buffer	1 mL x 1	-70°C
② 10X Nicotinamide	1 mL x 1	-70°C
③ 10X PRPP*	1 mL x 1	-70°C
④ 10X ATP	1 mL x 1	-70°C
⑤ recombinant NAMPT**	200 μL x 1	-70°C
⑥ recombinant NMNAT1***	200 μL x 1	-70°C
⑦ 50X WST-1	200 μL x 1	-70°C
⑧ 50X ADH (alcohol dehydrogenase)	200 μL x 1	-70°C
⑨ 50X Diaphorase	200 μL x 1	-70°C
⑩ 10X EtOH	1 mL x 1	-70°C
⑪ Instruction manual	1	room temp.

* Phosphoribosyl pyrophosphate
** Human NAMPT (nicotinamide phosphoribosyltransferase) expressed in *E. coil*.
*** Human NMNAT1 (nicotinamide mononucleotide adenylyltransferase 1) expressed in *E. coil*.

Materials Required but not Provided

- **Microplate for ELISA**
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **Pipettors:** 2-20 μL, 20-200 μL and 200-1000 μL precision pipettors with disposable tips.
- **Multi-channel pipette**
- **Microplate shaker**
- **Deionized water of the highest quality**
- **500 or 1000 mL graduated cylinder**
- **Reagent reservoirs**
- **FK866 (APO866):** FK866 is available from Axon Medchem Cat.# Axon 1279 or Cayman Cat.# 13287. Make 1 mM stock solution in DMSO (Optional).
- **NAD+:** NAD+ (β -Nicotinamide adenine dinucleotide hydrate) is from Sigma Cat.# N7004. Prepare fleshly 5 μM solution in H_2O from 1 mM stock solution in H_2O. Discard any unused 5 μM NAD+ (Optional).

For Reference Purpose Only!

Precautions

- Please thaw all reagents in crushed ice before use.

- Please avoid freezing-and-thawing cycle of ⑤recombinant NAMPT, ⑥recombinant NMNAT1, ⑧ 50x ADH and ⑨50x Diaphorase in this kit. There is a possibility that the enzymes may be inactivated. Aliquot to 25-50 µL and store at –70°C

- Please avoid mixing of **any reagents containing SH group like DTT or reduced glutathione, or alkyl amine** in the sample that will interfere this assay.

- Do not use kit components beyond the indicated kit expiration date.

- Rinse all detergent residue from glassware.

- Use deionized water of the highest quality.

- Do not mix reagents from different kits.

- Do not mouth pipette or ingest any of the reagents.

- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.

- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**

For Reference Purpose Only!

For Reference Purpose Only!

Detailed Protocol

Description of assay system

The CycLex NAMPT Colorimetric Assay Kit can measure the enzyme activity of nicotinamide phosphoribosyltransferase (NAMPT) by an enzyme-coupled reaction as shown in "Measuring Principle of The CycLex NAMPT Colorimetric Assay Kit" at page 2. In this method, NAMPT converts nicotinamide to nicotinamide mononucleotide (NMN), subsequently nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1) converts NMN to NAD+. Resultant NAD+ can be measured by enzyme cycling reaction using alcohol dehydrogenase (ADH), diaphorase and WST-1. Since the reaction is not stopped, it is necessary to monitor absorbance of WST-1-formazan at 450 nm at regular intervals after the reaction is initiated, and to determine reaction velocity.

The CycLex NAMPT Colorimetric Assay Kit can measure the enzyme activity of NAMPT with two kinds of measuring methods, the 1-Step Method and the 2-Step Method. The 1-Step Method is accomplished by mixing with four enzymes, i.e. NAMPT, NMNAT1, alcohol dehydrogenase and diaphorase. Since three coupled reactions are promoted simultaneously with NAMPT enzyme reaction, detection sensitivity of this method is less than that of 2-Step Method.

Conversely, the 2-Step Method is begun by initiating reactions of two enzymes, NAMPT, NMNAT1 within a set time period to produce NDA+ from nicotinamide and PRPP; then in the second step, followed by adding ADH, diaphorase and WST-1, the resultant WST-1-formazan is formed by NAD/NADH cycling enzyme reaction.

Preparation Method for Assay Reagents

Stand all reagents in ice to thaw. Use them after they thaw completely.

#1. 1-Step Assay Buffer (Quantity Required: 60 µL/assay)

・Mix following reagents and put in ice.

・This 1-Step Assay Buffer should be used within 30 min after prepared. Discard any unused 1-Step Assay Buffer after use.

Reagents	Volume
① 10X NAMPT assay buffer	10 µL
② 10X Nicotinamide	10 µL
③ 10X PRPP	10 µL
④ 10X ATP	10 µL
⑥ recombinant NMNAT1	2 µL
⑦ 50X WST-1	2 µL
⑧ 50X ADH	2 µL
⑨ 50X Diaphorase	2 µL
⑩ 10X EtOH	10 µL
dH_2O	2 µL
Total	60 µL

#2. 2-Steps Assay Buffer-I (Quantity Required: 90 µL/assay)

・Mix following reagents and put in ice.
・This 2-Step Assay Buffer-I should be used within 30 min after prepared. Discard any unused 2-Step Assay Buffer-I after use.

Assay reagents	Volume
① 10X NAMPT assay buffer	10 µL
② 10X Nicotinamide	10 µL
③ 10X PRPP	10 µL
④ 10X ATP	10 µL
⑥ recombinant NMNAT1	2 µL
dH_2O	48 µL
Total	90 µL

#3. 2-Step Assay Buffer-II (Quantity Required: 20 µL/assay)

・Mix following reagents and put in ice.
・This 2-Step Assay Buffer-II should be used within 30 min after prepared. Discard any unused 2-Step Assay Buffer-II after use.

Assay reagents	Volume
⑦ 50X WST-1	2 µL
⑧ 50X ADH	2 µL
⑨ 50X Diaphorase	2 µL
⑩ 10X EtOH	10 µL
dH_2O	4 µL
Total	20 µL

For Reference Purpose Only!

NAMPT Assay Procedures

1. 1-Step Method

Assay reagents	Test sample	Positive control	No enzyme control
Your enzyme sample (Test sample)	**10 µL**	**-**	**-**
⑤ recombinant NAMPT	**-**	**2 µL**	**-**
dH_2O	**30 µL**	**38 µL**	**40 µL**
#1. 1-Step Assay Buffer (see page 5)	**60 µL**	**60 µL**	**60 µL**

1. Following the above table, add **10 µL of "Your enzyme sample"** or **2 µL of** ⑤ **recombinant NAMPT** and **dH_2O** to the well of microplate and mix well. Next, initiate reaction by adding **60 µL of #1. 1-Step Assay Buffer** to each well and mix thoroughly. Incubate at 30°C.

2. Monitor the absorbance at 450 nm for 60 min at 5 min intervals using microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

2. 2-Step Method

1st reaction; Conversion of nicotinamide to NAD

Assay reagents	Test sample	Positive control	No enzyme control
Your enzyme sample (Test sample)	**10 µL**	**-**	**-**
⑤ recombinant NAMPT	**-**	**2 µL**	**-**
dH_2O	**-**	**8 µL**	**10 µL**
#2. 2-Step Assay Buffer-I (see page 6)	**90 µL**	**90 µL**	**90 µL**

1. Following the above table, add **10 µL of "Your enzyme sample"** or **2 µL of** ⑤ **recombinant NAMPT** and **dH_2O** to each well of the microplate. Finally, initiate reaction by adding **90 µL of #2. 2-Step Assay Buffer-I** to each well and mix thoroughly to initiate reaction. Incubate at 30°C for 60 min.

2nd reaction; Measurement of NAD

2. Add **20 µL of #3. 2-Step Assay Buffer-II** (see page 6) to each well of the microplate and mix thoroughly. Incubate at 30°C.

3. Monitor the absorbance at 450 nm for 30 min at 5 min intervals using microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

For Reference Purpose Only!

3. Special considerations when screening inhibitors or activators

In order to estimate the inhibitory effect on NAMPT enzyme activity in the test chemicals correctly, it is necessary to conduct the control experiment of "Vehicle control" at least once for every experiment and "Inhibitor control" at least once for the first experiment, in addition to "Test chemical", as indicated in the following table. When test chemicals cause an inhibitory effect on NAMPT enzyme activity, the level of A450 is weakened as compared with "Vehicle control". The high level of A450 is not observed in "Inhibitor control" (usually A450<0.2).

I. 1-Step Method

Assay reagents	Test chemical	Vehicle control	Inhibitor control
⑤ recombinant NAMPT	2 µL	2 µL	2 µL
50X Inhibitor candidate	2 µL	-	-
Vehicle for Inhibitor candidate	-	2 µL	-
FK866 (1 mM)*	-	-	2 µL
dH_2O	36 µL	36 µL	36 µL
#1. 1-Step Assay Buffer	60 µL	60 µL	60 µL

* FK866 (1 mM): Not provided in this kit. See page 3.

1. Following the above table, add **2 µL of ⑤ recombinant NAMPT**, **dH_2O** and **50X Inhibitor candidate** or **Vehicle for Inhibitor candidate** or **FK866 (1 mM)** to each well of microplate and mix well. Next, initiate reaction by adding **60 µL** of **#1. 1-Step Assay Buffer** to each well and mix thoroughly. Incubate at 30°C.

2. Monitor the absorbance at 450 nm for 60 min at 5 min intervals using microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

II. 2-Step Method

Assay reagents	Test chemical	Vehicle control	Inhibitor control
⑤ recombinant NAMPT	2 µL	2 µL	2 µL
50X Inhibitor candidate	2 µL	-	-
Vehicle for Inhibitor candidate	-	2 µL	-
FK866 (1 mM)	-	-	2 µL
dH_2O	6 µL	6 µL	6 µL
#2. 2-Step Assay Buffer-I	90 µL	90 µL	90 µL

For Reference Purpose Only!

* FK866 (1 mM): Not provided in this kit. See page 3.

1. Following the above table, add **2 µL of ⑤ recombinant NAMPT**, **dH_2O** and **50X Inhibitor candidate** or **Vehicle for Inhibitor candidate** or **FK866 (1 mM)** to each well of the microplate. Next, initiate reaction by adding **90 µL** of **#2. 2-Step Assay Buffer-I** to each well and mix thoroughly to initiate reaction. Incubate at 30°C for 60 min.

2. Add **20 µL of #3. 2-Step Assay Buffer-II** to each well of the microplate and mix thoroughly. Incubate at 30°C.

3. Monitor the absorbance at 450 nm for 30 min at 5 min intervals using microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

For Reference Purpose Only!

4. Assay control

I. For Inhibitor screening

Since the CycLex NAMPT Colorimetric Assay Kit can measure the NAMPT enzyme activity by an enzyme-coupled reaction, in which four enzymes involved, including NAMPT, NMNAT1, ADH and diaphorase, when test chemicals that have an inhibitory effect on one of these enzymes, the signal will be reduced. If there is such a possibility, please carry out the experiment of NAD/NADH recycling assay in the following Table and NMNAT1 assay using CycLex NMNAT1 Colorimetric Assay Kit (CY-1252) to ascertain which enzyme is target of the test chemical.

Assay reagents	Test chemical	Vehicle control
NAD+ (5 µM)*	**10 µL**	**10 µL**
50X Inhibitor candidate	**2 µL**	**-**
Vehicle for Inhibitor candidate	**-**	**2 µL**
dH_2O	**68 µL**	**68 µL**
#3. 2-Step Assay Buffer-II	**20 µL**	**20 µL**

* NAD+: Not provided in this kit. See page 3.

1. Following the above table, add, **10 µL of NAD+ (5 µM)**, **dH_2O** and **50X Inhibitor candidate** or **Vehicle for Inhibitor candidate** to each well of microplate and mix well. Next, initiate reaction by adding **20 µL** of **#3. 2-Step Assay Buffer-II** to each well and mix thoroughly. Incubate at 30°C.

2. Monitor the absorbance at 450 nm for 60 min at 5 min intervals using microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

When there is an inhibitory effect of test chemical on NAD/NADH recycling reaction, A450 will not increase.

For Reference Purpose Only!

II. For NAMPT activity assay in an immunoprecipitate

Since NAD+ level in cells is relatively high, around several hundreds micromolar concentration, NAD+ might mix easily in purified NAMPT from various cells or an immunoprecipitate using the specific antibody against NAMPT. Such contaminated NAD+ in the test sample causes false positive result by initiating NAD/NADH cycling enzyme reaction. If there is such a possibility, please carry out the experiment of NAD/NADH recycling assay in the following Table.

Assay reagents	Test sample	NAD+ control
Your enzyme sample (Test sample)	**10 µL**	**-**
NAD+ (5 µM)	**-**	**10 µL**
dH_2O	**70 µL**	**70 µL**
#3. 2-Step Assay Buffer-II	**20 µL**	**20 µL**

* NAD+: Not provided in this kit. See page 3.

1. Following the above table, add **10 µL of "Your enzyme sample"** or **10 µL of NAD+ (5 µM)** and **dH_2O** to each well of microplate and mix well. Next, initiate reaction by adding **20 µL** of **#3. 2-Step Assay Buffer-II** to each well and mix thoroughly. Incubate at 30°C.

2. Monitor the absorbance at 450 nm for 60 min at 5 min intervals using microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

When there is contaminated NAD+ in the test sample, A450 will increase in "Test sample".

For Reference Purpose Only!

III. For NAMPT activity assay in an immunoprecipitate (Alternative method)

In order to measure the activity of NAMPT correctly, it is necessary to conduct the control experiments for "**No enzyme control**" and "**No Nicotinamide control**" at least once in addition to "**Your enzyme sample**," as indicated in the below table of assay method. Although A450 increases in **"Test sample**" when NAMPT enzyme activity is in the sample, the increase in A450 is not observed in "**No enzyme control**" and "**No Nicotinamide control**".

Assay reagents	Test sample	Positive control	No enzyme control	No Nicotinamide control
① **10X NAMPT assay buffer**	**10 µL**	**10 µL**	**10 µL**	**10 µL**
② **10X Nicotinamide**	**10 µL**	**10 µL**	**10 µL**	**-**
③ **10X PRPP**	**10 µL**	**10 µL**	**10 µL**	**10 µL**
④ **10X ATP**	**10 µL**	**10 µL**	**10 µL**	**10 µL**
⑥ **recombinant NMNAT1**	**2 µL**	**2 µL**	**2 µL**	**2 µL**
#3. 2-Step Assay Buffer-II (see page 6)	**20 µL**	**20 µL**	**20 µL**	**20 µL**
dH2O	**28 µL**	**36 µL**	**38 µL**	**38 µL**
Your enzyme sample (Test sample)	**10 µL**	**-**	**-**	**10 µL**
⑤ **recombinant NAMPT**	**-**	**2 µL**	**-**	**-**

1. Following the above table, add all reagents (① **10X NAMPT Assay Buffer** to **dH$_2$O)** to each well of microplate and mix well. Next, initiate reaction by adding **10 µL of "Your enzyme sample"** or **2 µL of ⑤ recombinant NAMPT** to each well and mix thoroughly. Incubate at 30°C.

2. Monitor the absorbance at 450 nm for 60 min at 5 min intervals using microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

The difference in the reaction velocity between "**Test sample**" and "**No Nicotinamide control**" indicates the NAMPT activity.

For Reference Purpose Only!

Evaluation of Results

Analysis of Kinetics

Time course curve

1. Run reactions as described in the Detailed Protocol.
2. Subtract A450 at the 0 time from all reaction time points.
3. Plot A450 versus reaction time.
4. Determine the reaction time range in which the increase in A450 is linear.
5. Calculate activity:

$$\text{Activity (reaction velocity)} = \frac{\text{A450 of Test Sample}}{\text{Reaction time (min.)}}$$

NOTE: Usually, the linear range is from 20 to 40 min. This value is variable depending on reaction conditions and storage/handling of the Recombinant NAMPT. Decreasing the amount of Recombinant NAMPT in the assay may help to lengthen the time range.

Cautions

1. Since this kit is based on NAD+ detection system, it is not possible to detect NAMPT activity in crude cell extract in which NAD+ concentration is relatively high. **Please use an immunoprecipitate using the specific antibody against NAMPT* as a test sample**.

   *CycLex recommends Anti-Human NAMPT Mouse Monoclonal Antibody (Cat# CY-M1035)

2. Contaminated NAD+ in the test sample causes false positive result by initiating NAD/NADH cycling enzyme reaction. Please confirm no NAD+ in the test sample according to "4. Assay control", page 11 or 12.
3. Duplicate measurement is strongly recommended for accurate measurement of NAMPT activity.

4. Although we suggest to conduct experiments as outlined in "Protocol for immunoprecipitation" at page 15, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.

For research use only, not for use in diagnostic or therapeutic procedures

For Reference Purpose Only!

Assay Characteristics

The CycLex Research Product **CycLex NAMPT Assay kit** has been shown to detect the activity of nicotinamide phosphoribosyltransferase activity in recombinant NAMPT or an immunoprecipitate using the specific antibody against NAMPT. The assay shows good linearity of sample response. The assay may be used to follow the purification of NAMPT.

Troubleshooting

1. When test chemicals have an inhibitory effect on NMNAT1, ADH or diaphorase, precise inhibitory effect on NAMPT enzyme activity cannot be measured.

2. The recombinant NAMPT should be run in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.

3. Poor duplicates indicate inaccurate dispensing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for multi-channel pipettor maintenance.

Reagent Stability

All of the reagents included in the CycLex Research Product **CycLex NAMPT Assay kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. **All kit components should be stored at -70°C.**

For Reference Purpose Only!

Protocol for immunoprecipitation

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, 1 mM DTT and protease inhibitor cocktail.
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 the 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).
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20

Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing test compound for desired time.

2. To harvest cells under non-denaturing 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 to each plate (10 cm dish) 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 –70°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 and add 1-2 µg of anti-NAMPT antibody* which can be used for immunoprecipitation, incubate with gentle rocking for 2 hrs or overnight at 4°C.
   *CycLex recommend Anti-Human NAMPT Mouse Monoclonal Antibody (Cat# CY-M1035)

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 10 seconds at 4°C. Wash the beads twice 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 and measure NAMPT activity according to "NAMPT Assay Procedures", page 7.

For Reference Purpose Only!

Example of Test Results

Fig.1 Dose dependency curve of recombinant NAMPT activity using 1-Step Method

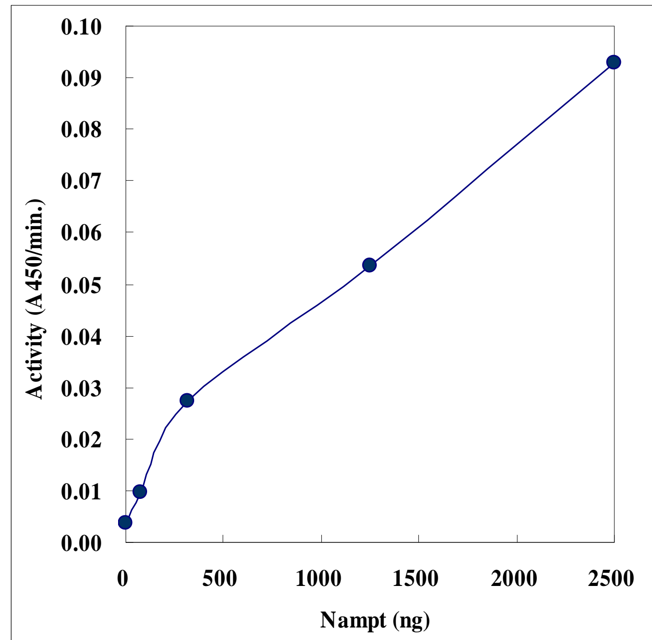
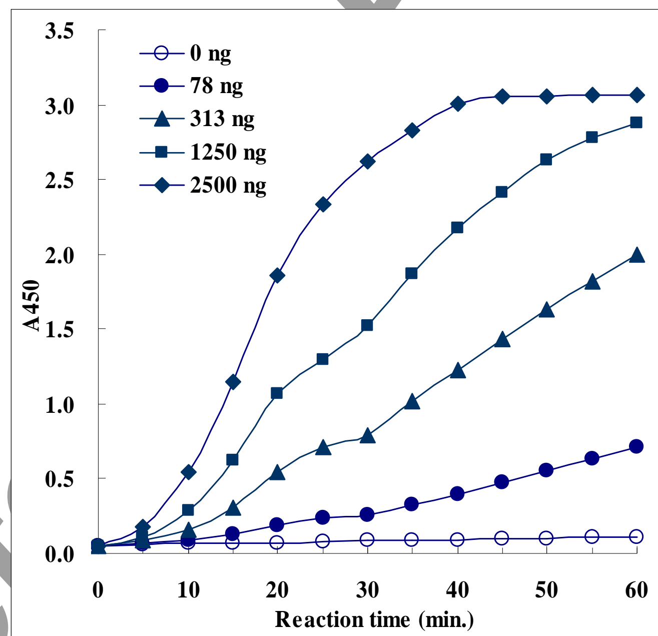


Fig.2 Time course of NAMPT activity in recombinant NAMPT using 1-Step Method



For Reference Use Only!

Fig.3 Effect of FK866 on recombinant NAMPT activity

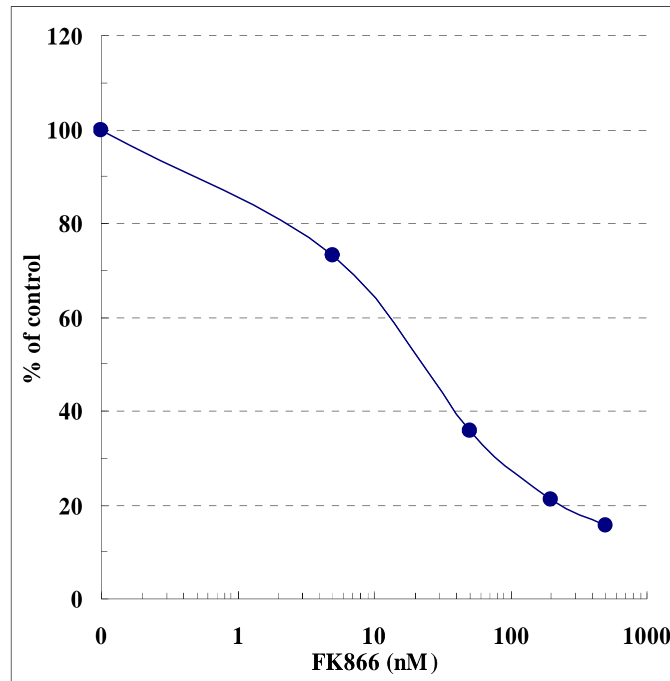
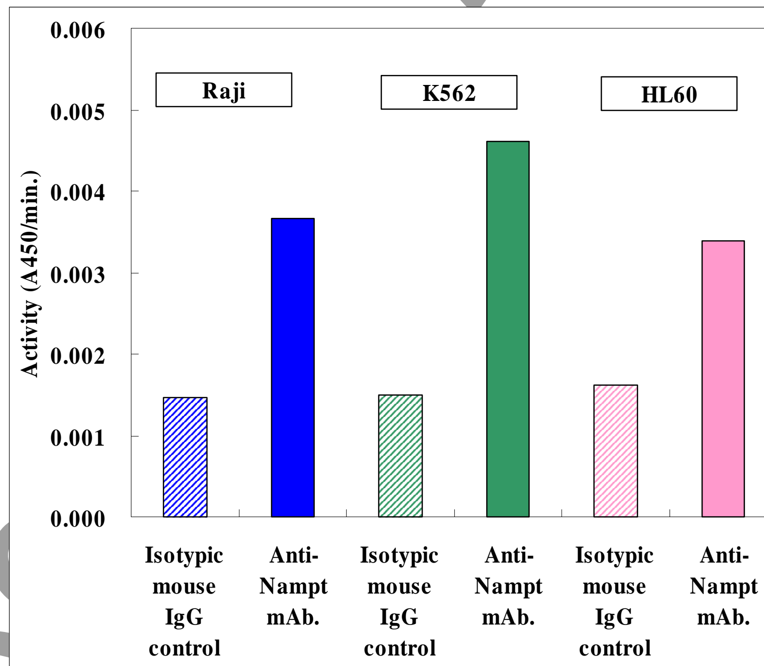


Fig.4 Measurement of endogenous NAMPT activity in an immunoprecipitate using anti-Human NAMPT Mouse Monoclonal Antibody (CY-M1035) from extracts of three cell lines



For Research Purpose Only!

Fig.5 Measurement of NAMPT activity in the immunoprecipitate from cell extract of 293T cells, which had been transfected with NAMPT expression plasmid DNA

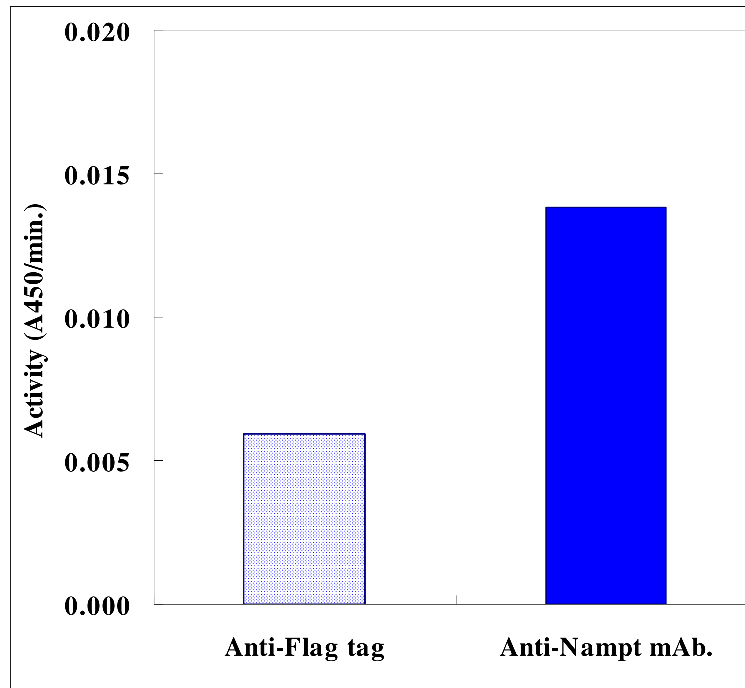
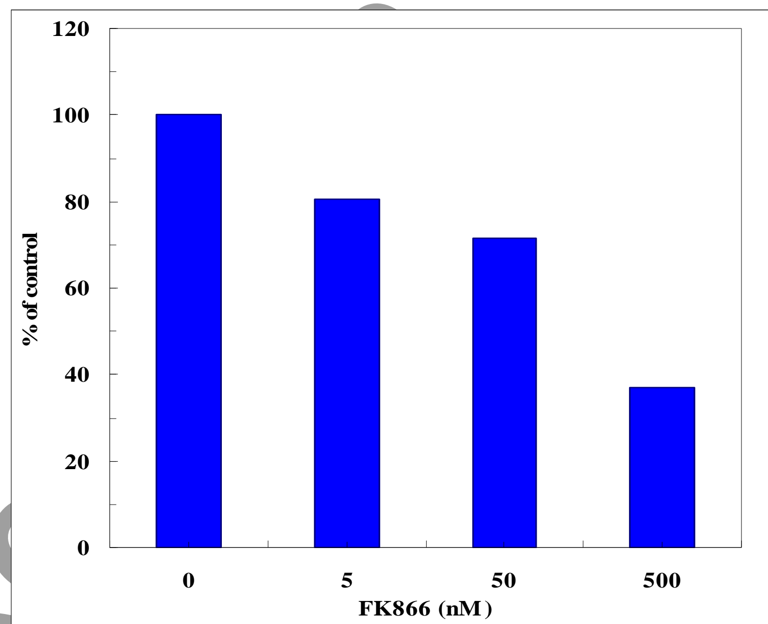


Fig.6 Effect of FK866 on NAMPT activity in the immunoprecipitate from Raji cells extract using anti-Human NAMPT Mouse Monoclonal Antibody (CY-M1035)



For Research Purpose Only!

Fig.7 Effect of FK866 on NAMPT activity in activity in the immunoprecipitate using anti-Flag antibody from cell extract of 293T cells, which had been transfected with Flag-NAMPT expression plasmid DNA

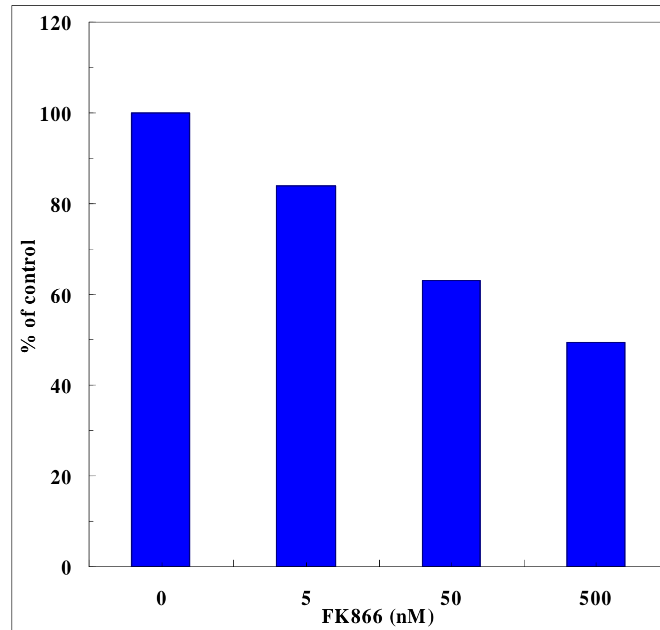
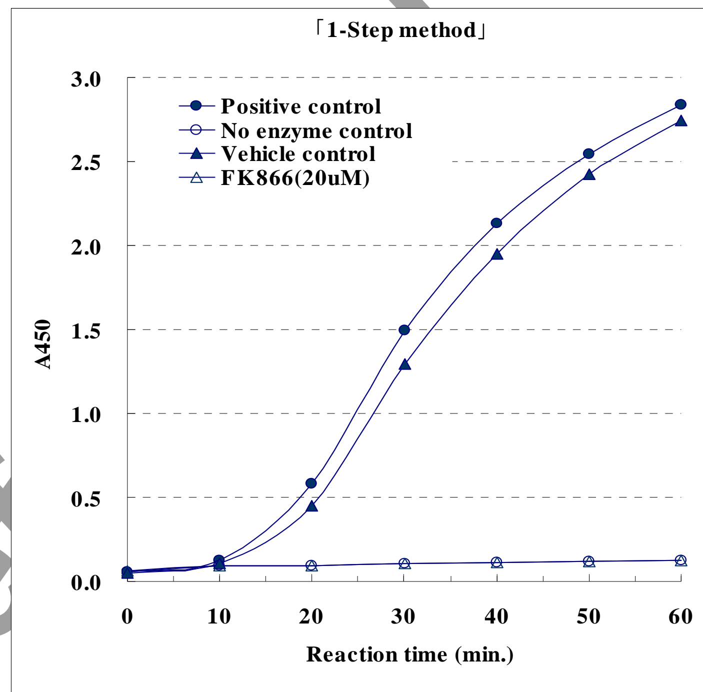


Fig.8 Typical data of 1-Step Method according to section "3. Special considerations when screening inhibitors or activators" at page 8



For Research Purpose Only!

Fig.9 Typical data of 2-Step Method according to section "3. Special considerations when screening inhibitors or activators" at page 9

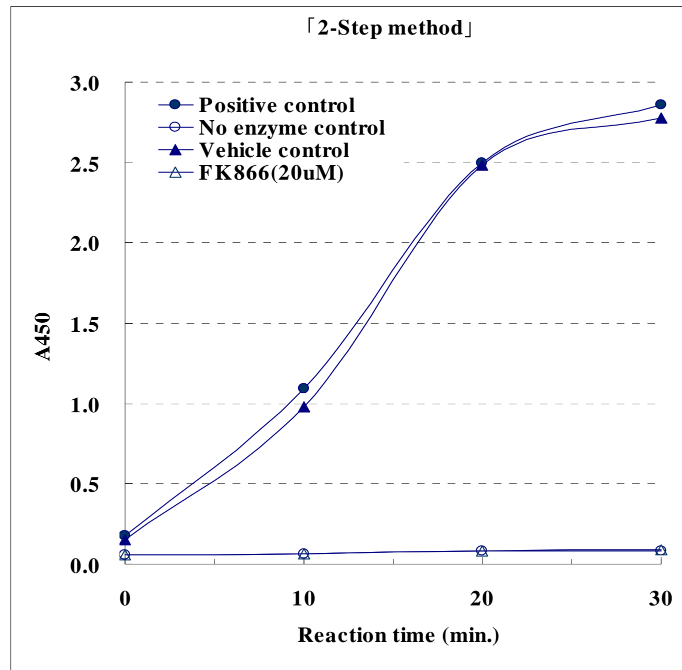
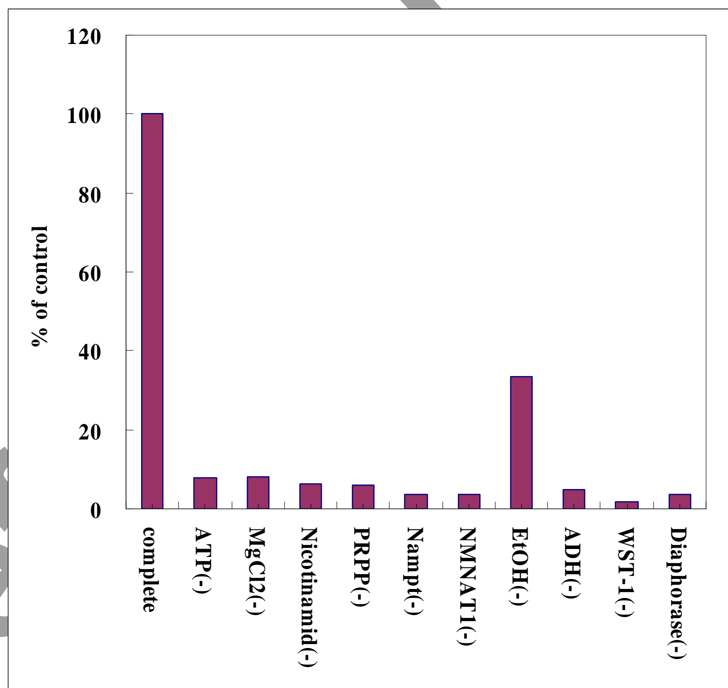


Fig.10 Requirement of each assay component for measurement of NAMPT activity



For Research Purpose Only!

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

For Reference Purpose Only!

Related Products

* CycLex NAMPT Colorimetric Assay Kit: Cat# CY-1251
* CycLex NMNAT Colorimetric Assay kit: Cat# CY-1252
* NAMPT (Nicotinamide Phosphoribosyltransferase): Cat# CY-E1251
* NMNAT1 (Nicotinamide Mononucleotide Adenylyltransferase 1): Cat# CY-E1252
* CycLex SIRT1/Sir2 Deacetylase Fluorometric Assay Kit: Cat# CY-1151
* CycLex SIRT2 Deacetylase Fluorometric Assay Kit: Cat# CY-1152
* CycLex SIRT3 Deacetylase Fluorometric Assay Kit: Cat# CY-1153
* CycLex SIRT6 Deacetylase Fluorometric Assay Kit: Cat# CY-1156
* NAD(+)-Dependent Deacetylase SIRT1: Cat# CY-E1151
* NAD(+)-Dependent Deacetylase SIRT2: Cat# CY-E1152
* NAD(+)-Dependent Deacetylase SIRT3: Cat# CY-E1153
* NAD(+)-Dependent Deacetylase SIRT6: Cat# CY-E1156

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

CycLex/CircuLex products are supplied for research use only. CycLex/CircuLex products and components thereof may not be resold, modified for resale, or used to manufacture commercial products without prior written approval from CycLex Co., Ltd.. To inquire about licensing for such commercial use, please contact us via email.

For Reference Purpose Only!