For Research Use Only, Not for use in diagnostic procedures

Quantitative Assay for Nicotinamide Mononucleotide Adenylyltransferase Activity

CycLex NMNAT1 Colorimetric Assay Kit Ver.2

For 100 assays

Cat# CY-1252V2

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Intended Use

The MBL Research Product **CycLex NMNAT1 Colorimetric Assay Kit Ver.2** detects nicotinamide mononucleotide adenylyltransferase (NMNAT) activity in recombinant NMNAT1 or endogenous NMNAT family members immunoprecipitated from cell lysates. Primarily, the **CycLex NMNAT1 Colorimetric Assay Kit Ver.2** is designed for the rapid and sensitive evaluation of NMNAT1 inhibitors or activators using recombinant NMNAT1. Since this kit is based on NAD^+ detection system, it is impossible to directly detect NMNAT activity in crude cell lysates in which NAD^+ concentration is relatively high.

Individual users should determine appropriate conditions when using other types of samples.

Applications for this kit include:
1) Screening inhibitors or activators of NMNAT1.
2) Detecting effects of pharmacological agents on NMNAT1.
3) Measuring NMNAT activity of immunoprecipitated or highly purified NMNAT family members.

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.
- Do not expose reagents to excessive light.

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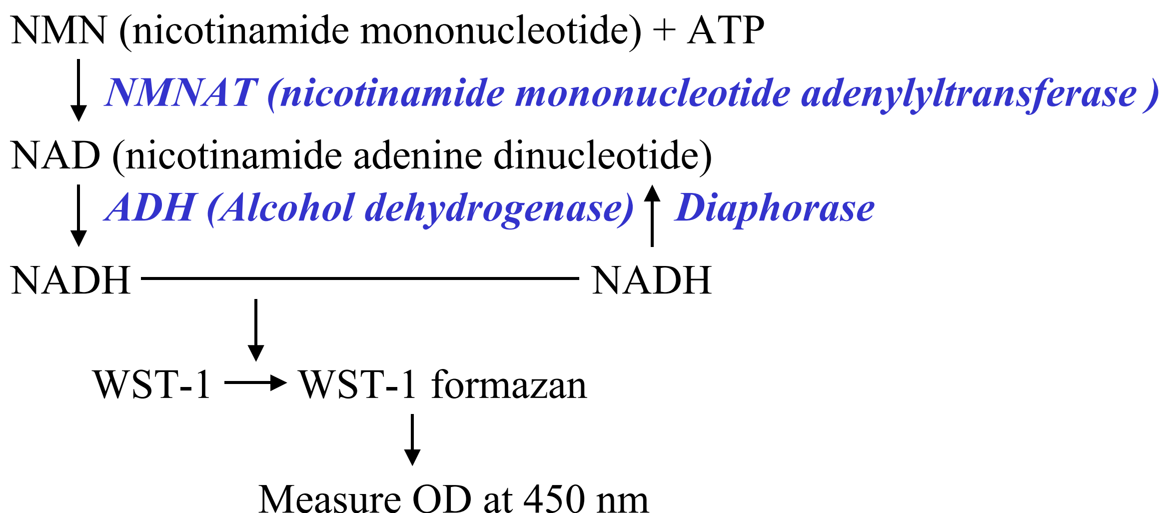
Introduction

Nicotinamide mononucleotide adenylyltransferase (NMNAT) is a central enzyme in NAD^+ biosynthesis, transferring the adenylyl moiety of ATP to β-Nicotinamide mononucleotide (NMN) or nicotinic acid mononucleotide (NaMN) resulting in the formation of NAD^+ or NaAD and the release of pyrophosphate. As this reaction is reversible, the enzyme may in principle be used to form ATP and NMN from NAD^+ and pyrophosphate. This enzyme could be a potential target for therapeutical applications, because its activity is rather low in tumor cells. The deduced protein contains an N-terminal nuclear localization signal. Immunofluorescence microscopy localized endogenous NMNAT1 to the nucleus in human fibroblasts and in a hepatoma cell line. It was demonstrated that NMNAT1 inhibited recombinant human poly(ADP-ribose) polymerase-1 by about 35%, and it completely prevented the formation of branched ADP-ribose polymers.

Principle of the Assay

The CycLex NMNAT1 Colorimetric Assay Kit Ver.2 can measure the enzyme activity of nicotinamide mononucleotide adenylyltransferase (NMNAT) by an enzyme-coupled reaction. In this method, NMNAT converts β-Nicotinamide mononucleotide (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.

Measuring Principle of the CycLex NMNAT1 Colorimetric Assay Kit Ver.2



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Materials Provided

Components of the Kit

Components	Quantity	Storage
#1. NMNAT Assay Buffer (20X)	1 mL x 1	-70°C
#2. WST-1	500 µL x 1	-70°C
#3. ADH	500 µL x 1	-70°C
#4. Diaphorase	500 µL x 1	-70°C
#5. EtOH Solution	500 µL x 1	-70°C
#6. ATP	500 µL x 1	-70°C
#7. NMNAT1*	500 µL x 1	-70°C
#8. NMN	500 µL x 1	-70°C
Instruction manual	1	Room temp.

* Human NMNAT1 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 200-1,000 µL precision pipettors with disposable tips.
- **Multi-channel pipette**
- **Microplate shaker**
- **The highest quality deionized (distilled) water (ddH$_2$O)**
- **500 or 1,000 mL graduated cylinder**
- **Reagent reservoirs**
- **(Optional) NAD$^+$:** β-Nicotinamide adenine dinucleotide hydrate, available from Sigma Cat# N7004. Prepare fleshly 10 µM solution in ddH$_2$O from 1 mM stock solution. Discard any unused 5 µM NAD$^+$.
- **(Optional) Gallotannin (Tannic acid):** A potent inhibitor of Nicotinamide mononucleotide adenylyltransferase (NMNAT), available from Sigma Cat# T3437. Prepare 0.2 mM solution in ddH$_2$O.

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Precautions

- **Although we suggest to conduct experiments as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.**
- Thaw all reagents completely in crushed ice before use.
- Avoid repeated freezing and thawing of the recombinant NMNAT1 in this kit. There is a possibility that the enzyme activity may be inactivated. Aliquot to 25-50 µL and store at -70°C
- Avoid mixing of **any reagents containing SH group like DTT or reduced glutathione**, **or** **alkyl amine** in samples that will interfere this assay.
- Do not use a kit beyond the indicated the 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.**

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Detailed Protocol

The MBL Research Product **CycLex NMNAT1 Colorimetric Assay Kit Ver.2** can measure the enzyme activity of NMNAT1 with two different measuring methods, One-Step Assay Method and Two-Step Assay Method. One-Step Assay Method is accomplished by mixing with three enzymes, i.e. NMNAT1, alcohol dehydrogenase (ADH) and diaphorase. Since two coupled reactions are promoted simultaneously with NMNAT1 enzyme reaction, detection of this method is less sensitive than the Two-Step Assay Method.

Conversely, Two-Step Assay Method is begun by initiating reactions of NMNAT1 within a set time period to produce NAD^+ from NMN and ATP. Then in the second step, followed by adding ADH, diaphorase and WST-1, the resultant WST-1-formazan is formed by NAD^+/NADH enzyme cycling reaction.

1. Preparation of Assay Reagents

1) Place all components on ice to thaw. Use them after thawing and vortexing completely.
   * If use the One-Step Assay Method, go to 2). If use the Two-Step Assay Method, go to 2').

2) For One-Step Assay Method, prepare **One-Step Assay Mixture** (Quantity required: 60 µL/assay). Mix following components and put on ice. Use within 30 minutes after preparation. Discard any unused One-Step Assay Mixture after use.

One-Step Assay Mixture

Components	Volume
#1. NMNAT Assay Buffer (20X)	**5 µL**
#2. WST-1	**5 µL**
#3. ADH	**5 µL**
#4. Diaphorase	**5 µL**
#5. EtOH Solution	**5 µL**
#6. ATP	**5 µL**
#8. NMN	**5 µL**
ddH_2O	**25 µL**
Total	**60 µL**

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2') For Two-Step Assay Method, prepare **Two-Step Assay Mixture-I** (Quantity required: 60 µL/assay) and **Two-Step Assay Mixture-II** (Quantity required: 20 µL/assay). Mix following components and put on ice. Use within 30 minutes after preparation. Discard any unused Two-Step Assay Mixture-I and Mixture-II after use.

Two-Step Assay Mixture-I

Components	Volume
#1. NMNAT Assay Buffer (20X)	**5 µL**
#6. ATP	**5 µL**
#8. NMN	**5 µL**
ddH$_2$O	**45 µL**
Total	**60 µL**

Two-Step Assay Mixture-II

Components	Volume
#2. WST-1	**5 µL**
#3. ADH	**5 µL**
#4. Diaphorase	**5 µL**
#5. EtOH Solution	**5 µL**
Total	**20 µL**

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2. Assay Methods for Measurement of NMNAT Activity

I. One-Step Assay Method

Assay reagents	Enzyme Sample Assay	No Enzyme Control Assay	Positive Control Assay
ddH_2O	**35 µL**	**35 µL**	**35 µL**
Enzyme Sample*	**5 µL**	**-**	**-**
Buffer of Enzyme Sample*	**-**	**5 µL**	**-**
#7. NMNAT1	**-**	**-**	**5 µL**
One-Step Assay Mixture	**60 µL**	**60 µL**	**60 µL**
Total Volume of the Reaction	**100 µL**	**100 µL**	**100 µL**

* The addition volume can be changed with adjusting the one of ddH_2O.

1) Following the table above, add **ddH_2O**, and your **Enzyme Sample**, **Buffer of Enzyme Sample**, or **#7. NMNAT1** to each well of the microplate.

2) Initiate the reaction by adding **60 µL** of **One-Step Assay Mixture** to each well and mix thoroughly.

3) Incubate at 30°C and monitor the absorbance at 450 nm for 60 minutes at 5 minute intervals using a microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

II. Two-Step Assay Method

1st Reaction: Conversion of nicotinamide to NAD^+

Assay reagents	Enzyme Sample Assay	No Enzyme Control Assay	Positive Control Assay
ddH_2O	**15 µL**	**15 µL**	**15 µL**
Enzyme Sample*	**5 µL**	**-**	**-**
Buffer of Enzyme Sample*	**-**	**5 µL**	**-**
#7. NMNAT1	**-**	**-**	**5 µL**
Two-Step Assay Mixture-I	**60 µL**	**60 µL**	**60 µL**
Total Volume of the 1st Reaction	**80 µL**	**80 µL**	**80 µL**

* The addition volume can be changed with adjusting the one of ddH_2O.

1) Following the table above, add **ddH_2O** and your **Enzyme Sample** or **Buffer of Enzyme Sample** or **#7. NMNAT1** to each well of the microplate.

2) Initiate the 1st reaction by adding **60 µL** of **Two-Step Assay Mixture-I** to each well and mix thoroughly.

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3) Incubate at 30°C for 60 minutes.

2nd Reaction: Measurement of generated NAD^+

4) Add **20 µL** of **Two-Step Assay Mixture-II** to each well of the microplate and mix thoroughly.

5) Incubate at 30°C and monitor the absorbance at 450 nm for 30 minutes at 5 minute intervals using a microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

3. Special Considerations When Screening Inhibitors

In order to correctly estimate the inhibitory effect on NMNAT1 enzymatic activity, it is necessary to conduct the control experiments for "Solvent Control Assay" and "Inhibitor Control Assay" at least once for the first experiment, in addition to "Test Compound Assay", as indicated in the following procedure.

I. One-Step Assay Method

Assay reagents	Test Compound Assay	Solvent Control Assay	Inhibitor Control Assay
ddH_2O	**30 µL**	**30 µL**	**30 µL**
#7. NMNAT1	**5 µL**	**5 µL**	**5 µL**
20X Test Compound* Solvent for Test Compound* Gallotannin (2 mM)**	**5 µL - -**	**- 5 µL -**	**- - 5 µL**
One-Step Assay Mixture	**60 µL**	**60 µL**	**60 µL**
Total Volume of the Reaction	**100 µL**	**100 µL**	**100 µL**

* The addition volume can be changed with adjusting the one of ddH_2O.
** Not provided in this kit. See the section "Materials Required but not Provided" above.

1) Following the table above, add **ddH_2O**, **#7. NMNAT1**, and your **20X Test Compound**, **Solvent for Test Compound**, or **Gallotannin (2 mM)** to each well of microplate and mix well.

2) Initiate the reaction by adding **60 µL** of **One-Step Assay Mixture** to each well and mix thoroughly.

3) Incubate at 30°C and monitor the absorbance at 450 nm for 60 minutes at 5 minute intervals using a microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

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II. Two-Step Assay Method

1st Reaction: Conversion of Nicotinamide to NAD^+

Assay reagents	Test Compound Assay	Solvent Control Assay	Inhibitor Control Assay
ddH_2O	**10 µL**	**10 µL**	**10 µL**
#7. NMNAT1	**5 µL**	**5 µL**	**5 µL**
20X Test Compound* Solvent for Test Compound* Gallotannin (2 mM)**	**5 µL - -**	**- 5 µL -**	**- - 5 µL**
Two-Step Assay Mixture-I	**60 µL**	**60 µL**	**60 µL**
Total Volume of the 1st Reaction	**80 µL**	**80 µL**	**80 µL**

* The addition volume can be changed with adjusting the one of ddH_2O.
** Not provided in this kit. See the section "Materials Required but not Provided" above.

1) Following the table above, add **ddH_2O**, **#7. NMNAT1**, and your **20X Test Compound**, **Solvent for Test Compound**, or **Gallotannin (2 mM)** to each well of the microplate.

2) Initiate the reaction by adding **60 µL** of **Two-Step Assay Mixture-I** to each well and mix thoroughly.

3) Incubate at 30°C for 60 minutes.

2nd Reaction: Measurement of the generated NAD^+

4) Add **20 µL** of **Two-Step Assay Mixture-II** to each well of the microplate and mix thoroughly.

5) Incubate at 30°C and monitor the absorbance at 450 nm for 30 minutes at 5 minute intervals using a microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

Note: If your Test Compound causes an inhibitory effects on NMNAT1 enzyme activity, the level of the absorbance at 450 nm of the "Test Compound Assay" is weakened as compared with the one of the "Solvent Control Assay".

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4. Control Assay

I. For Inhibitor Screening

The CycLex NMNAT1 Colorimetric Assay Kit Ver.2 measures the NMNAT1 enzyme activity by an enzyme-coupled reaction in which three enzymes, i.e. NMNAT1, ADH, and diaphorase, are involved. If test compounds have an inhibitory effect on one of the enzymes, the signal will be reduced. If there is such a possibility, carry out the experiment of NAD^+/NADH enzyme cycling reaction according to the following procedure.

Assay reagents	Test Compound Assay	Solvent Control Assay
ddH_2O	**70 µL**	**70 µL**
NAD^+ (10 µM)*	**5 µL**	**5 µL**
20X Test Compound** **Solvent for Test Compound****	**5 µL** **-**	**-** **5 µL**
Two-Step Assay Mixture-II	**20 µL**	**20 µL**
Total Volume of the Reaction	**100 µL**	**100 µL**

* Not provided in this kit. See the section "Materials Required but not Provided" above.
** The addition volume can be changed with adjusting the one of ddH_2O.

1) Following the table above, add, **ddH_2O**, **NAD^+ (10 µM)**, and your **20X Test compound** or **Solvent for Test compound** to each well of microplate and mix well.

2) Initiate the reaction by adding **20 µL** of **Two-Step Assay Mixture-II** to each well and mix thoroughly.

3) Incubate at 30°C and monitor the absorbance at 450 nm for 60 minutes at 5 minute intervals using microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

Note: If there is an inhibitory effect of your Test Compound on NAD^+/NADH enzyme cycling reaction, the absorbance at 450 nm will not increase in the "Test Compound Assay".

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II. for Checking NAD^+ contamination in Immunoprecipitate

Since NAD^+ level in cells is relatively high, around several hundred micromolar concentration, NAD^+ might mix easily with purified NMNAT family members from various cells or an immunoprecipitate using the specific antibodies against NMNAT family members. Such contaminated NAD^+ in the enzyme sample causes a false positive result by initiating NAD^+/NADH enzyme cycling reaction. If there is such a possibility, carry out the experiment of NAD^+/NADH enzyme cycling reaction in the following procedure.
The related MBL Research Product CycLex NAD^+/NADH Colorimetric Assay Kit Ver.2 (Cat# CY-1253V2) can be also used for and will facilitate this experiment.

Assay reagents	Enzyme Sample Assay	NAD^+ Control Assay
ddH_2O	**75 µL**	**75 µL**
Enzyme Sample **NAD^+ (10 µM)***	**5 µL** **-**	**-** **5 µL**
Two-Step Assay Mixture-II	**20 µL**	**20 µL**
Total Volume of the Reaction	**100 µL**	**100 µL**

* Not provided in this kit. See the section "Materials Required but not Provided" above.

1) Following the table above, add **ddH_2O**, and your **Enzyme Sample** or **NAD^+ (10 µM)** to each well of microplate and mix well.

2) Initiate the reaction by adding **20 µL** of **Two-Step Assay Mixture-II** to each well and mix thoroughly.

3) Incubate at 30°C and monitor the absorbance at 450 nm for 60 minutes at 5 minute intervals using a microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

Note: If there is NAD^+ contamination in your Enzyme Sample, the level of the absorbance at 450 nm of the "Enzyme Sample Assay" will increase as compared with the one of the "NAD^+ Control Assay".

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III. for NMNAT Activity Assay with Immunoprecipitate

In order to measure the activity of NMNAT correctly, it is necessary to conduct the control experiments for "No Enzyme Control Assay" and "No NMN Control Assay" at least once in addition to "Enzyme Sample Assay" and "Positive Control Assay", as indicated in the following procedure.

Assay reagents	Enzyme Sample Assay	Positive Control Assay	No Enzyme Control Assay	No NMN Control Assay
ddH_2O	**60 µL**	**60 µL**	**65 µL**	**65 µL**
#1. NMNAT Assay Buffer (20X)	**5 µL**	**5 µL**	**5 µL**	**5 µL**
#6. ATP	**5 µL**	**5 µL**	**5 µL**	**5 µL**
#8. NMN	**5 µL**	**5 µL**	**5 µL**	**-**
Two-Step Assay Mixture-II	**20 µL**	**20 µL**	**20 µL**	**20 µL**
Enzyme Sample* **#7. NMNAT1**	**5 µL** **-**	**-** **5 µL**	**-** **-**	**5 µL** **-**
Total Volume of the Reaction	**100 µL**	**100 µL**	**100 µL**	**100 µL**

* The addition volume can be changed with adjusting the one of ddH_2O.

1) Following the table above, add all reagents (**ddH_2O** to **Two-Step Assay Mixture-II)** to each well of microplate and mix well.

2) Initiate the reaction by adding **5 µL** of your **Enzyme Sample** or **#7. NMNAT1** to each well and mix thoroughly.

3) Incubate at 30°C and monitor the absorbance at 450 nm for 30 minutes at 5 minute intervals using a microtiter plate reader. Measure and calculate the rate of reaction while the reaction velocity remains constant.

Note-1: The difference in the reaction velocity between the "Enzyme Sample Assay" and "No NMN Control Assay" indicates the actual NMNAT activity.

Note-2: If there is NAD^+ contamination in your Enzyme Sample, the level of the absorbance at 450 nm of the "No NMN Control Assay" will increases as compared with the one of the "No Enzyme Control Assay".

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Evaluation of Results

Analysis of Kinetics

Time course curve

1. Run reactions as described in the section **"Detailed Protocol"**.
2. Subtract the absorbance at 450 nm (A450) of the "No Enzyme Control Assay" from the one of the "Enzyme Sample Assay" at all reaction time points.
3. Plot the A450 versus reaction time.
4. Determine the reaction time range in which the increase of the A450 is linear.
5. Calculate activity:

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

Note: Usually, the linear range is from 20 to 40 minutes. This value is variable depending on reaction conditions and storage/handling of the recombinant NMNAT1. Decreasing the amount of recombinant NMNAT1 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 NMNAT activity in crude cell extract in which NAD^+ concentration is relatively high. **To use an immunoprecipitate as an enzyme sample, using the specific antibodies against NMNAT family members is recommended.**

2. Contaminated NAD^+ in the enzyme sample causes a false positive result by initiating NAD^+/NADH enzyme cycling reaction. Confirm no NAD^+ in the enzyme sample according to the **"4. Control Assay"** in the section **"Detailed Protocol"** above.

3. Duplicate measurement is strongly recommended for accurate measurement.

4. Although we suggest to conduct experiments as outlined in the section **"Protocol for Immunoprecipitation"** below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by individual users.

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Assay Characteristics

The MBL Research Product **CycLex NMNAT1 Colorimetric Assay Kit Ver.2** has been shown to detect the activity of nicotinamide phosphoribosyltransferase in recombinant NMNAT1 and an immunoprecipitate using the specific antibodies against NMNAT family members. The assay shows good linearity of sample response. The assay may be used to follow the purification of NMNAT family members.

Troubleshooting

1. If test compounds have an inhibitory effect on ADH or diaphorase, precise inhibitory effect on NMNAT1 enzyme activity cannot be measured.

2. All samples, standards and controls should be assayed 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 MBL Research Product **CycLex NMNAT1 Colorimetric Assay Kit Ver.2** have been tested for stability. The kit should not be used beyond the stated expiration date.

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Protocol for Immunoprecipitation

Immunoprecipitation Followed by Measuring NMNAT Activity

Note: Although we suggest to conduct experiments as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.

Solutions and Reagents

Cell Lysis Buffer: 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.

1X NMNAT Assay Buffer: Dilute "#1. NMNAT Assay Buffer (20X)" 20-fold with ddH_2O.

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 and spin down. Wash the pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)

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 of 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, the lysate can be stored at -70°C.

Immunoprecipitation

1. Take 250 µL of 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 1-2 µg of specific antibodies against NMNAT family members which can be used for immunoprecipitation, and incubate with gentle rocking for 2 hours or 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.

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5. Microcentrifuge for 10 seconds at 4°C. Wash the beads twice with 500 µL of ice-cold Cell Lysis Buffer, subsequently twice with 500 µL of ice-cold 1X NMNAT Assay Buffer. Keep on ice during washes.

6. Resuspend the beads with 20 µL of ice-cold 1X NMNAT Assay Buffer and measure NAMPT activity according to the "Detailed Protocol" above.

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Example of Test Results

Fig.1 Time course of recombinant NMNAT1 activity using One-Step Assay Method

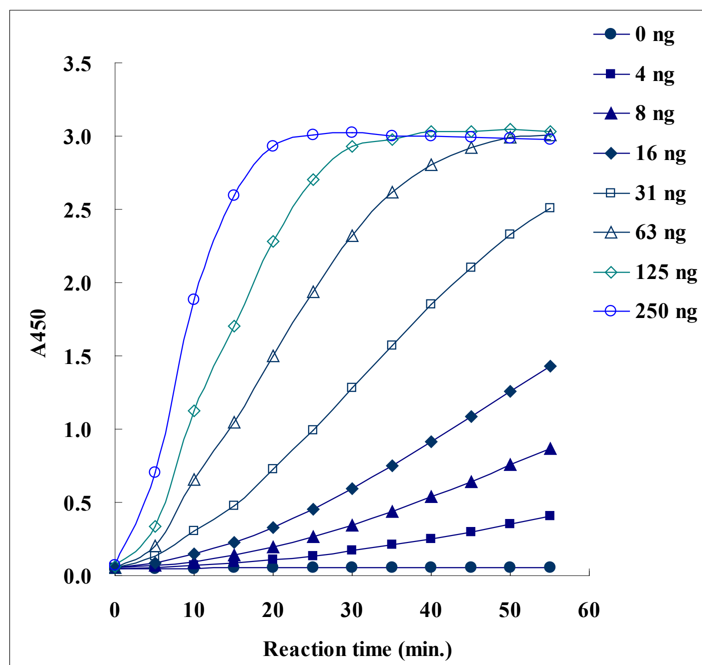

Fig.2 Dose dependent curve of recombinant NMNAT1 activity using One-Step Assay Method

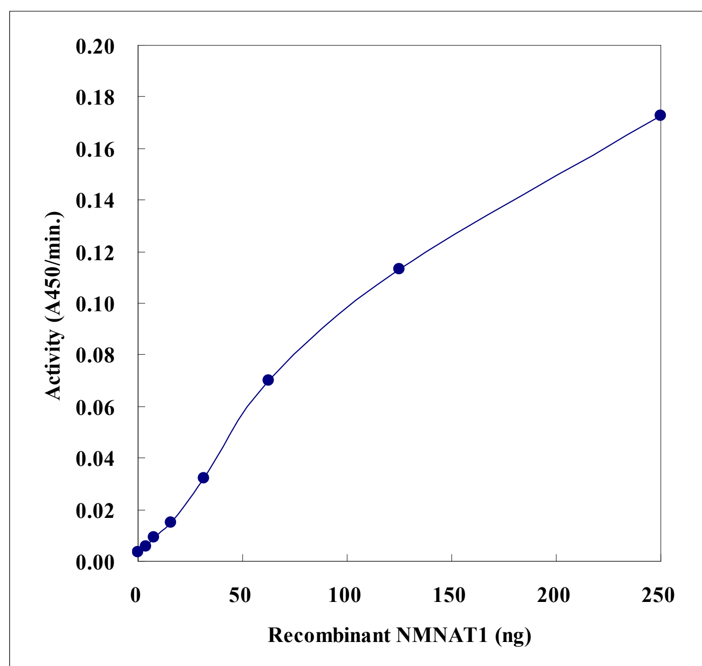

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Fig.3 Inhibitory effect of Gallotannin on recombinant NMNAT1 activity (10 min. after)

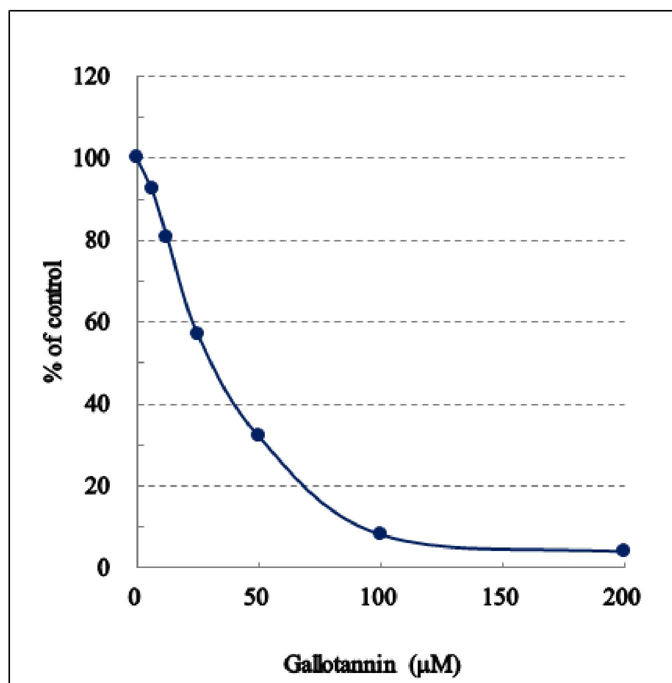

Fig.4 Measurement of endogenous NMNAT1 activity in an immunoprecipitate using anti-human NMNAT1 rabbit polyclonal antibody from lysates of Raji cells

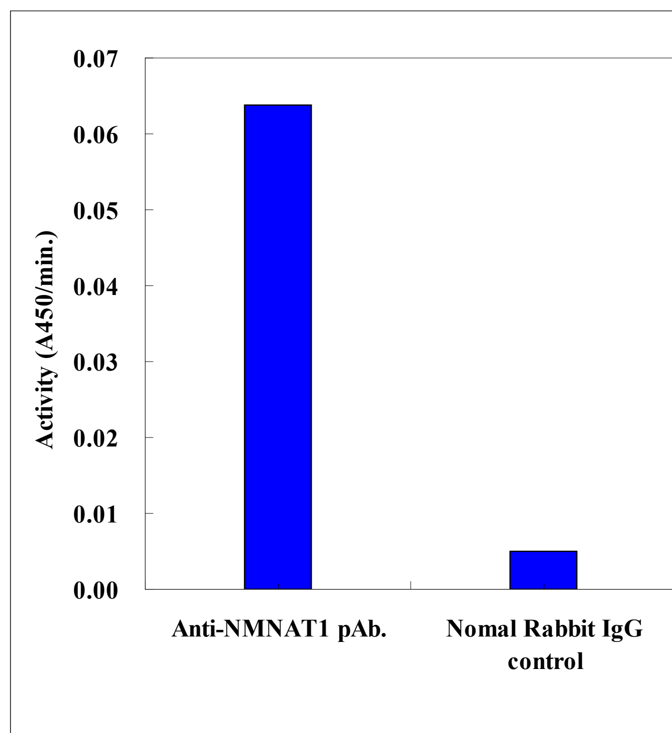

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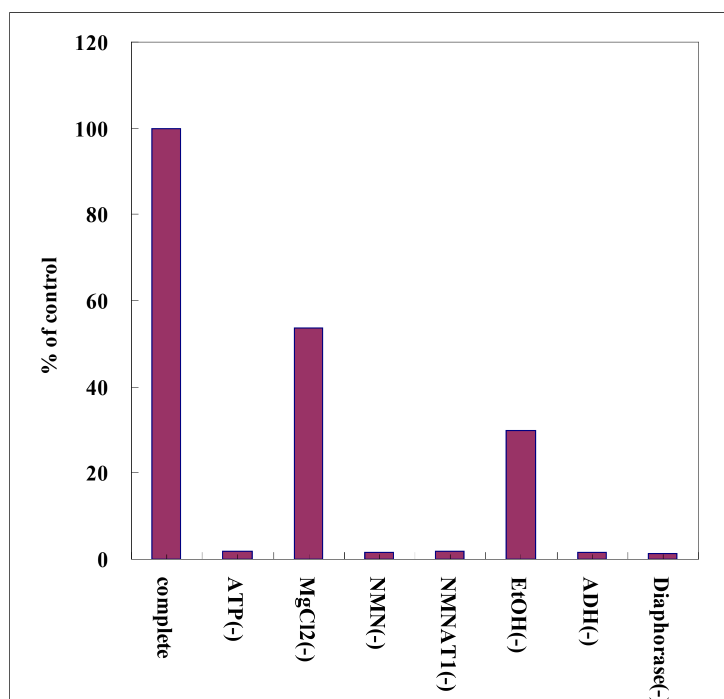
Fig.5 Requirement of each assay component for measurement of NMNAT1 activity



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