



NAMPT Activity Assay Kit

Catalog Number EA-7062

(For Research Use Only)

Introduction

Nicotinamide phosphoribosyltransferase (NAMPT) is a crucial enzyme involved in the biosynthesis of NAD^+ , a vital coenzyme in cellular metabolism. NAMPT has been implicated in various biological processes, including inflammation, cellular metabolism, and cancer.

Principle of the assay

The NAMPT activity assay kit can measure the activity of NAMPT for compound screening and profiling experiments. This assay detects NAMPT activity through a series of enzyme reactions. First, NAMPT converts nicotinamide (NAM) and PRPP into nicotinamide mononucleotide (NMN). Then NMN is converted by NMNAT into NAD^+ . Lastly, NAD^+ is reduced to NADH by alcohol dehydrogenase (ADH) in the presence of ethanol. The NADH is detected with a WST solution which reacts with NADH to product a yellow color that can be measured at an absorbance of 450 nm with a plate reader.

Materials Provided

- 100mM ATP (-20°C)
- 10mM NAM (-20°C)
- 10mM PRPP (-20°C)
- 1x NMNAT Enzyme Stock (-80°C)
- ADH Buffer (RT)
- 1x ADH Enzyme Stock (-80°C)
- Mediator Reagent (-20°C)
- WST Reagent (-80°C)

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 μ L of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C .
6. Use PBS to dilute the cell sample to the appropriate concentration, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C .
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

Assay Procedure

1. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
100mM ATP	0.05 μ L
10mM NAM	0.05 μ L
10mM PRPP	0.05 μ L
1x NMNAT Enzyme	1 μ L
1x ADH Enzyme	0.05 μ L
ADH Buffer	49.95 μ L
Total	50 μ L

2. Any unused enzyme stock can be stored at -80°C for future use.
3. Add 50 μ L of reaction mix to each well of the plate.
4. Add 10-20 μ L of sample to each well with reaction mix and mix thoroughly.
5. Cover the plate and incubate at 37°C for 10 minutes.
6. Detection mix preparation: calculate the amount of each reagent needed to make the detection mix according to the table below.

Component	Detection Mix (per well/sample)
WST Reagent	5 μ L
Mediator Reagent	0.5 μ L
PBS	44.5 μ L
Total	50 μ L

7. Add 50 μ L of detection mix to each well in the plate.
8. Cover the plate and incubate at 37°C away from light for 30 minutes.
Exposure to light will produce background signal in wells
9. For a stronger signal, the plate can be incubated for an additional 15-30 minutes at 37°C away from light.
10. Measure the absorbance of the plate at 450 nm using a plate reader.