Signosis
Innovative Plate Assay Solutions

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

- NAM (-20°C)
- 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)

Sample preparation before starting experiment

- For **cell culture medium samples**, add 50µl directly to the well.
- For **cell lysate samples**, use lysis buffer. Cell Lysis Buffer for ELISA available (Catalog# EA-0001). Follow protocol in Cell Lysis Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X Diluent buffer, for example, add 80ul sample in 720ul 1X Diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

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)
NAM	1 µL
PRPP	1 µL
1x NMNAT Enzyme	0.05 µL
1x ADH Enzyme	0.05 µL
ADH Buffer	49.95 µL
Total	50 µL

2. Any unused enzyme stock can be stored at -80°C for future use.
3. Add 50 µL of reaction mix to each well of the plate.
4. Add 10 µL 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 µL
Mediator Reagent	0.5 µL
PBS	44.5 µL
Total	50 µL

7. Add 50 µL of detection mix to each well in the plate.
8. Cover the plate and incubate at 37°C away from light for 30-60 minutes. **Exposure to light will produce background signal in wells**
9. For a stronger signal, the plate can be incubated for an additional 1-2 hours at 37°C away from light.
10. Measure the absorbance of the plate at 450 nm using a plate reader.