Description

The NAMPT Inhibitor Screening Assay Kit is a 96-well fluorogenic assay designed to measure the activity of NAMPT (nicotinamide phosphoribosyltransferase) for screening and profiling applications. The kit contains enough purified NAMPT enzyme, NAMPT Assay Buffer, NAMPT Dilution Buffer, ATP, Nicotinamide, PRPP and ethanol for 100 enzyme reactions.

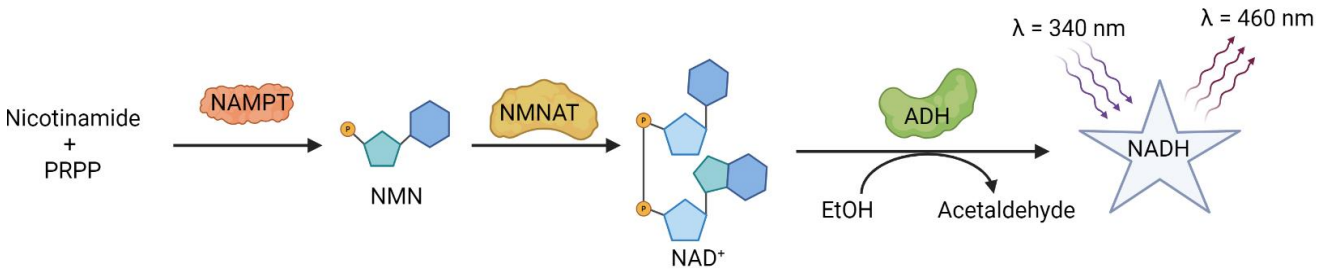


Figure 1 – Illustration of assay principle used in the NAMPT Inhibitor Screening Assay Kit.

This assay detects NAMPT activity through a series of three coupled reactions. NAMPT catalyzes the conversion of nicotinamide (NAM) and PRPP into nicotinamide mononucleotide (NMN). NMN is then converted by NMNAT into NAD^+, which is subsequently reduced to NADH by alcohol dehydrogenase (ADH) in the presence of ethanol. NADH emits fluorescence at 460 nm upon excitation at 340 nm. The intensity of this fluorescence correlates with the amount of NADH produced in the final step and it is directly proportional to the activity of NAMPT in the initial reaction.

Background

Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the formation of nicotinamide mononucleotide (NMN) from nicotinamide and 5-phosphoribosyl-1-pyrophosphate (PRPP). It is the rate limiting enzyme in the mammalian NAD (nicotinamide adenine dinucleotide) biosynthesis pathway. NAMPT is thought to be involved in many important biological processes, including metabolism, stress response, and aging. NAMPT is often overexpressed in cancer cells, an adaptation required to sustain the higher energetic need of the cells. In addition, it also plays roles in proliferation, invasion, stemness, angiogenesis, immune regulation, and drug resistance of tumors, making it an attractive target candidate for cancer therapy.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and High-Throughput Screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
91004-KC50	Human NAMPT (PBEF1), GST-Tag*	50 µg	-80°C
82663-KC3	NAMPT Dilution Buffer	3 ml	-80°C
79946-KC750	4x NAMPT Assay Buffer**	2 x 750 µl	-80°C
83720-KC250	400 µM ATP	250 µl	-80°C
82664-KC250	400 µM Nicotinamide	250 µl	-80°C
82665-KC250	800 µM PRPP	250 µl	-80°C
83523-KC1	30% Ethanol	1 ml	-80°C
79685	Low binding black microtiter 96-well plate	1	Room Temp.

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*
*** Thaw 4x NAMPT Assay Buffer on ice and do not vortex or sonicate.*

Materials Required but Not Supplied

- Fluorescent microplate reader capable of reading λexc/λem = 340 nm/460 nm
- Adjustable micropipettor and sterile tips
- Orbital Shaker
- 30°C Incubator

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone be tested to determine any potential interference of the compound with the assay results.
- Always maintain 4x NAMPT Assay Buffer on ice and do not vortex or sonicate.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.

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- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using FK-866 (#82682) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Thaw **4x NAMPT Assay Buffer, 400 µM ATP**, **400 µM Nicotinamide**, **800 µM PRPP, NAMPT Dilution Buffer** and **30% Ethanol** on ice.

   Note: The 4x NAMPT Assay Buffer contains NMNAT and ADH enzymes and must be kept on ice throughout the procedure. Do not vortex or sonicate and avoid freeze/thaw cycles.

2. Thaw **NAMPT** enzyme on ice. Briefly spin the tube to recover its full content.

3. Dilute NAMPT with NAMPT Dilution Buffer to 20-50 ng/µl (10 µl/well).

4. Add 10 µl of diluted NAMPT to the "Positive Control" and "Test Inhibitor" wells.

5. Add 10 µl of NAMPT Dilution Buffer to the "Blank" wells.

6. Prepare Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   6.1. If the Test Inhibitor is soluble in water, make a dilution in distilled water at a concentration 10-fold higher than the final desired concentration.

   For the positive and negative controls, use distilled water (Diluent Solution).

   OR

   6.2. If the Test Inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in distilled water to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in water so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO in the assay should not exceed 1%.

7. Add 5 µl of Test Inhibitor to the "Test Inhibitor" wells.

8. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.

9. Pre-incubate NAMPT with the inhibitors for 30 minutes at Room Temperature (RT), with gentle agitation.

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10. Prepare a **Master Mix** (35 µl/well): N wells x (12.5 µl of 4x NAMPT Assay Buffer + 2.5 µl of 400 µM ATP + 2.5 µl of 400 µM Nicotinamide + 2.5 µl of 800 µM PRPP + 2.5 µl of 30% Ethanol + 12.5 µl of distilled water).

11. Start the reaction by adding 35 µl of Master Mix to all wells.

12. Incubate at 30°C for 2 hours.

Component	Blank	Test Inhibitor	Positive Control
Diluted NAMPT (20-50 ng/µl)	-	10 µl	10 µl
NAMPT Dilution Buffer	10 µl	-	-
Test Inhibitor	-	5 µl	-
Diluent Solution	5 µl	-	5 µl
Incubate 30 minutes at RT			
Master Mix	35 µl	35 µl	35 µl
Total	**50 µl**	**50 µl**	**50 µl**

13. Measure the fluorescence intensity in a microtiter plate-reader fluorimeter capable of excitation at λ=340 nm and emission at λ=460 nm.

14. The "Blank" should be subtracted from all other values (background value).

Example Results

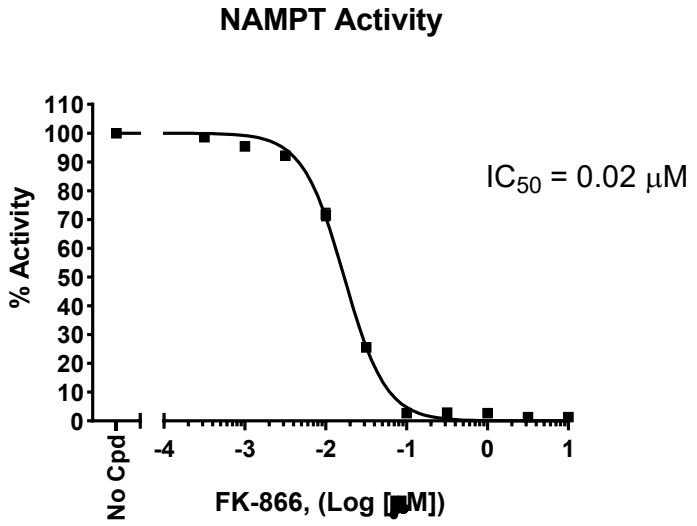


Figure 2: NAMPT inhibition by FK-866.
NAMPT activity was measured in the presence of increasing concentrations of FK-866 (#82682). Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

Data shown is representative.

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Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References

Ramsey K., *et al.*, 2009, *Science* 324 (5927): 651-4.
Nakahata Y., *et al.*, 2009, *Science* 324 (5927) : 654-7.
Gasparrini M., *et al.*, 2022, *Int J Biochem Cell Biol.* 145 : 106189.

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