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Data Sheet

NAMPT Inhibitor Screening Assay Kit

Catalog: 71276
Size: 96 reactions

BACKGROUND : NAMPT catalyzes the formation of nicotinamide mononucleotide from nicotinamide and 5-phosphoribosyl-1-pyrophosphate (PRPP). It is the rate limiting component in the mammalian NAD biosynthesis pathway. The protein is thought to be involved in many important biological processes, including metabolism, stress response and aging.

DESCRIPTION: The *NAMPT Inhibitor Screening Assay Kit* is designed to measure NAMPT activity for screening and profiling applications. The NAMPT assay kit comes in a convenient 96-well format, with purified recombinant NAMPT enzyme, NAMPT assay buffer, NAMPT dilution buffer, ATP, Nicotinamide, PRPP, and Ethanol sufficient for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
91004	NAMPT	50 µg	-80°C	***Avoid multiple freeze/thaw cycles!***
	NAMPT dilution buffer	3 ml	-80°C	
	4x NAMPT assay buffer	2 x 750 µl	-80°C	
79686	ATP (400 µM)	250 µl	-80°C	
	Nicotinamide (400 µM)	250 µl	-80°C	
	PRPP (800 µM)	250 µl	-80°C	
	Ethanol (30%)	1 ml	-80°C	
79685	Low binding black microtiter 96-well plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader
Adjustable micropipettor and sterile tips
Aluminum foil

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

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REFERENCE: Ramsey, K.M., et al. (2009) *Science* **324:** 651-654
Nakahata, Y., et al. (2009) *Science* **324:** 654-657.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1. Thaw **4x NAMPT assay buffer**, **ATP**, **Nicotinamide**, **Phosphoribosyl pyrophosphate (PRPP),** and **30% Ethanol** on ice.

2. Thaw **NAMPT dilution buffer** and **NAMPT** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of **NAMPT** required for the assay and dilute enzyme to 20-50 ng/µl with **NAMPT dilution buffer**. Aliquot remaining **NAMPT** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. ***NAMPT*** *enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3. Add 10 µl of diluted **NAMPT** enzyme to the wells designated “Positive Control” and "Test Inhibitor". To the wells designated as "Blank," add 10 µl of **NAMPT dilution buffer**.

4. Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the wells labeled “Positive Control" and “Blank,” add 5 µl of the same solution without inhibitor (Inhibitor buffer). *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO (aq). Then, add 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*

5. Preincubate **NAMPT** with the inhibitors for 30 min at room temperature (slow shaking).

6. During enzyme preincubation with the inhibitors, prepare the **Master Mixture** (35 µl per well): N wells x (12.5 µl **4x NAMPT assay buffer** + 2.5 µl **ATP** (400 µM) + 2.5 µl **Nicotinamide** (400 µM) + 2.5 µl **PRPP** (800 µM) + 2.5 µl 30% **Ethanol** + 12.5 µl **water**). Add 35 µl to every well. Store remaining components at -80°C in single use aliquots.

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	Positive Control	Test Inhibitor	Blank
4x NAMPT assay buffer	12.5 µl	12.5 µl	12.5 µl
ATP (400 µM)	2.5 µl	2.5 µl	2.5 µl
Nicotinamide (400 µM)	2.5 µl	2.5 µl	2.5 µl
PRPP (800 µM)	2.5 µl	2.5 µl	2.5 µl
30% Ethanol	2.5 µl	2.5 µl	2.5 µl
Water	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	–	5 µl	–
NAMPT dilution buffer in 10% DMSO (Inhibitor buffer)	5 µl	–	5 µl
NAMPT dilution buffer	–	–	10 µl
NAMPT (20-50 ng/µl)	10 µl	10 µl	–
Total	50 µl	50 µl	50 µl

7. Initiate the reaction by adding 35 µl of **Master Mixture** to all the wells. Incubate at 30°C for two hours.

8. After the reaction, read sample in a microtiter-plate reading fluorimeter capable of excitation at a wavelength of ~340 nm and detection of emitted light of ~460 nm. “Blank” value is subtracted from all other values.

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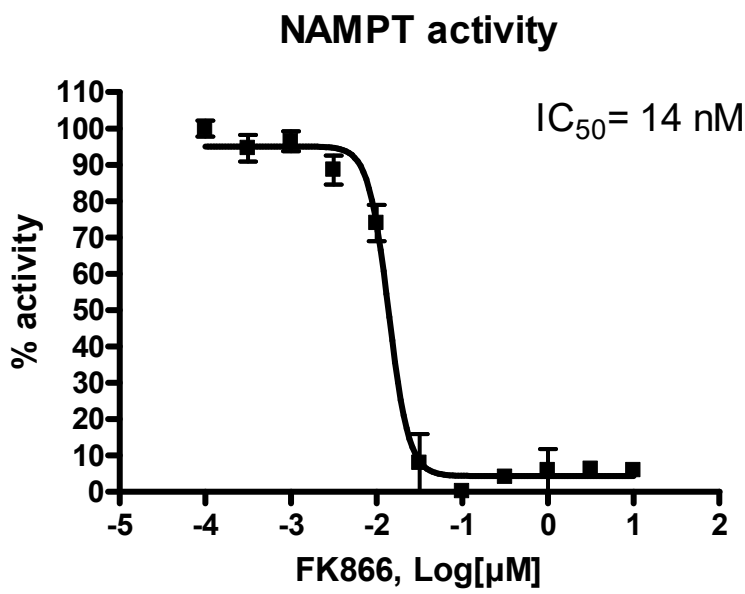
Example of Assay Results:



NAMPT inhibition by FK866, measured using the NAMPT Inhibitor Screening Assay Kit, BPS Bioscience Cat.71276. The compound was pre-incubated with the NAMPT enzyme for 30 min at room temperature before the reaction was initiated with the addition of master mix. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
NAMPT (PBEF1), GST tag	91004	50 µg
NAMPT (PBEF1)	71098	50 µg
NMNAT, His-tag	71090	100 µg
CD38, His-Tag (Human), HiP™	71227	100 µg
CD38 Inhibitor Screening Assay Kit	71275	96 rxns
5'-Nucleotidase/CD73, His-tag	71184	50 µg
CD73 Inhibitor Screening Assay Kit	72055	96 rxns
CD73 Inhibitor Screening Assay Kit	72058	384 rxns
Adenosine Deaminase (ADA), His-tag	70016	100 µg
NAD+, Biotin-Labeled	80610	500 µl