Description

The NNMT Fluorogenic Assay Kit is designed to measure NNMT (Nicotinamide N-methyltransferase) activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough recombinant NNMT, substrate, cofactor, and assay buffer for 100 enzyme reactions.

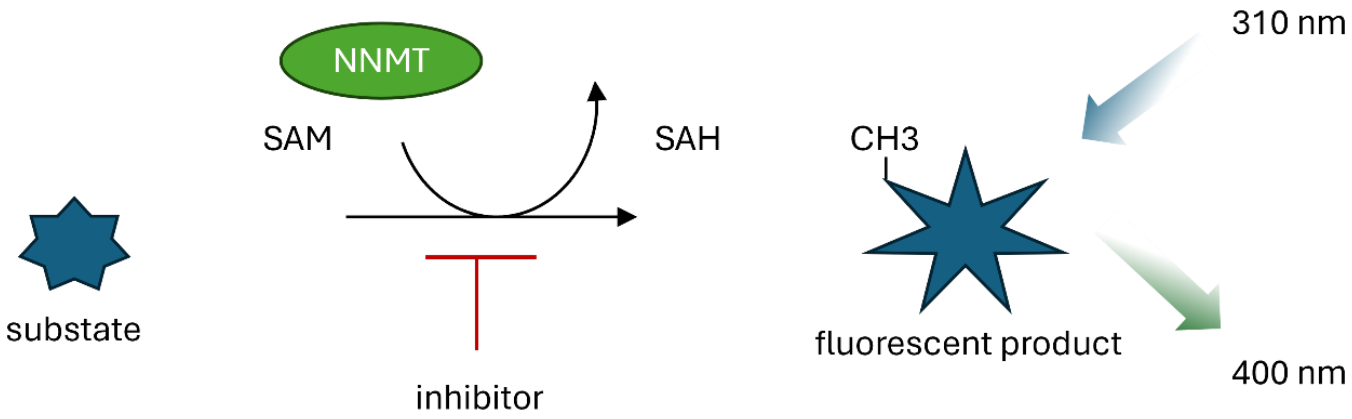

Figure 1: Illustration of the mechanism behind the NNMT Fluorogenic Assay Kit.
NNMT is incubated with a substrate and S-adenosyl-L-methionine (SAM) as the methyl donor. Fluorescence intensity increases proportionally to the activity of NNMT.

Background

NNMT (nicotinamide N-methyltransferase) is a metabolic enzyme involved in the N-methylation of nicotinamide to MNA (1-methylnicotinamide), using SAM (S-adenosylmethionine) as the donor of the methyl group. It is found at high levels in the liver, and at lower levels in the brain, kidney, adipose tissue thyroid and pancreas. NAD^+, an essential metabolite for generation of ATP by the mitochondrial respiration chain, and as substrate of proteins that include PARP1 [poly(ADP-ribose) polymerase 1] and sirtuins, is synthetized from tryptophan in the kynurenine pathway and from nicotinamide in a process that involves NAMPT (nicotinamide phosphoribosyltransferase) and NMNAT (nicotinamide adenine mononucleotide adenylyltransferase). The consumption of nicotinamide by NNMT has thus an impact on NAD^+ production. NNMT is overexpressed in several diseases, including Alzheimer's and Parkinson's disease, atherosclerosis, obesity, fatty liver disease, COPD (chronic obstructive pulmonary disease), and cancer. Abnormal levels of NNMT have an impact on the methylation of the genome by reducing the amount of SAM available, and thus play an epigenic role. Studies *in vitro* indicate that NNMT can support tumor growth by decreasing apoptosis and necrosis and increasing ATP production. NNMT plays an oncogenic role and promotes drug resistance, not because it is essential for cancer development but by providing cancer cells with an advantage versus normal cells. NNMT has thus become an attractive therapeutical target for combinatory therapy, with investigation focusing on the discovery of small molecules targeting either its activity or its expression levels. NNMTi, an NNMT inhibitor, was able to reverse high-fat diet induced obesity in an animal model and the tumor burden in an ovarian cancer metastasis one, showing the potential of inhibiting NNMT. The development of NNMT inhibitors with higher selectivity and potency is going to open new avenues of treatment in oncology as a support for other therapies.

Applications

Study enzyme kinetics and screen small molecule inhibitors of NNMT for drug discovery and high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
11357	NNMT, His-Tag Recombinant*	30 µg	-80°C
	NNMT Substrate	30 µl	-80°C
	250 µM S-adenosylmethionine (SAM)	400 µl	-80°C
	5X NNMT Assay Buffer	5 ml	-20°C
	0.5 M DTT	200 µl	-20°C
79685	Black 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Fluorimeter capable of excitation at λ=310 nm (10 nm bandwidth) and detection at λ=400 nm (10 nm bandwidth)
- 37°C incubator
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

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 is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

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- We recommend using JBSNF-000088 (#82631) 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.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Add 10 µl of 0.5 M DTT to 1 ml of 5x NNMT Assay Buffer.

2. Prepare 1x NNMT Assay Buffer by adding 1 volume of 5x NNMT Assay Buffer with DTT to 4 volumes of distilled water.

3. Thaw NNMT on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

4. Dilute NNMT to 15 ng/µl with 1x NNMT Assay Buffer (20 µl/well).

5. Prepare the **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.

   5.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in 1x NNMT Assay Buffer.

   For the positive and negative controls, use 1x NNMT Assay Buffer (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x NNMT Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using 1x NNMT Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in 1x NNMT Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

6. Add 20 µl of diluted NNMT to the “Positive Control” and “Test Inhibitor” wells.

7. Add 20 µl of 1x NNMT Assay Buffer to the “Blank” wells.

8. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

9. Add 5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

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10. Incubate at Room Temperature (RT) for 1 hour with gentle agitation.

11. Thaw NNMT Substrate and S-adenosylmethionine (SAM) on ice. Briefly spin the tubes to recover the full contents.

12. Prepare a Master Mix (25 µl/well): N wells x (22.25 µl of 1x NNMT Assay Buffer + 0.25 µl of NNMT Substrate + 2.5 µl of 250 µM S-adenosylmethionine (SAM)).

13. Add 25 µl of diluted Master Mix to every well. Protect your samples from direct exposure to light.

14. Incubate at 37°C for 1 hour.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Diluted NNMT (15 ng/µl)	-	20 µl	20 µl
NNMT Assay Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Pre-incubate for 1 hour at Room Temperature (RT)			
Master Mix	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

15. Read the plate in a fluorimeter capable of excitation at λ=310 nm (10 nm bandwidth) and detection at λ=400 nm (10 nm bandwidth).

16. The “Blank” value should be subtracted from all other readings.

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Example Results

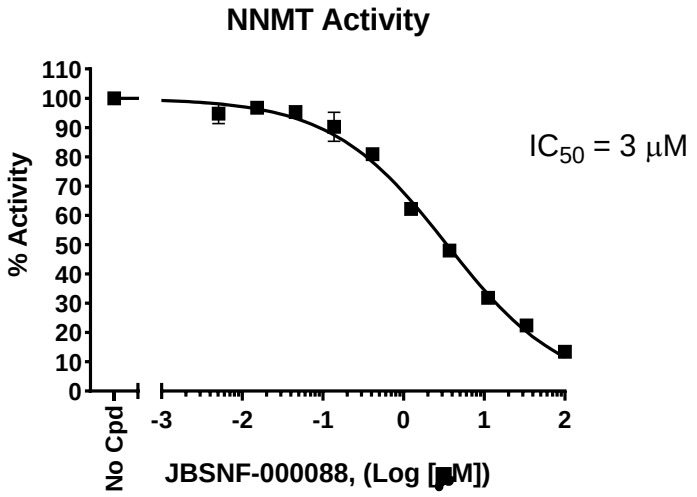

Figure 1: Inhibition of NNMT activity by the inhibitor JBSNF-000088.
NNMT activity was measured in the presence of increasing concentrations of JBSNF-000088 (#82631). The "Blank" value was subtracted from all other values.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Roberti A., *et al.*, 2021 *Molecular Metabolism* 45:101165.
Parons R. and Facey P., 2021 *Biomolecules* 11(10):1418.

Related Products

Products	*Catalog #*	*Size*
NMNAT1 Assay Kit	79642	96 reactions
NAMPT Inhibitor Screening Assay Kit	71276	96 reactions/384 reactions
NAD^+, Biotin-Labeled	80610	500 µl
400 µM S-Adenosylmethionine	52120	250 µl
NAMPT (PBEF1), GST-tag Recombinant	91004	50 µg
NMNAT1, His-Tag Recombinant	71090	100 µg

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