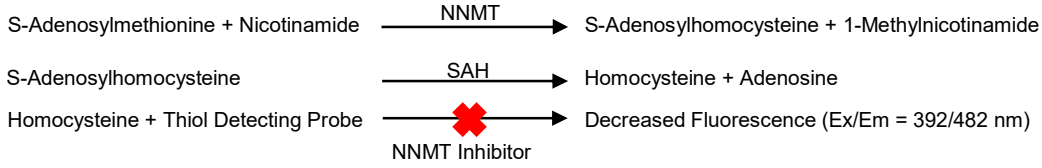
N′-Nicotinamide Methyltransferase (NNMT) Inhibitor Screening Assay

(Catalog # BN01039; 100 assays; Store at -80°C)

I. Introduction:

N′-Nicotinamide Methyltransferase (NNMT) (E.C. 2.1.1.1) catalyzes the N-methylation of nicotinamide, pyridines, and other analogues using S-adenosyl methionine (SAM) as the donor resulting in the production of 1-methylnicotinamide (MNA). NNMT plays a significant role in the regulation of metabolic pathways and is expressed at markedly high levels in several kinds of cancers, neurodegenerative diseases, obesity and diabetes, indicating it is a potential molecular target for therapy. Assay Genie's NNMT inhibitor screening kit utilizes SAM as the methyl group donor and nicotinamide as the substrate. NNMT methylates nicotinamide generating S-adenosylhomocysteine (SAH) and 1-methylnicotinamide. The SAH is hydrolyzed by SAH hydrolase to form homocysteine, the free thiol group of which is detected using a Thiol Detecting Probe generating enhanced fluorescence signal that can be measured at Ex/Em = 392/482 nm. In the presence of an NNMT inhibitor, the enzymatic activity is inhibited resulting in decreased fluorescence. This assay kit is a simple, sensitive, and rapid tool to screen potential inhibitors of NNMT.



II. Applications:

Screening/studying/characterizing potential inhibitors of NNMT

III. Kit Contents:

Components	BN01039	Cap Code	Part Number
NNMT Assay Buffer	22 ml	WM	BN01039-1
NNMT Enzyme	50 µl	Green	BN01039-2
S-Adenosylmethionine (SAM)	4 x Lyophilized	Yellow	BN01039-3
Nicotinamide	3 x 1.5 ml	Orange	BN01039-4
Enzyme-I	200 µl	Blue	BN01039-5
Enzyme-II	Lyophilized	Clear	BN01039-6
1-Methylnicotinamide (MNA) (150 mM)	20 µl	Red	BN01039-7
Thiol Detecting Probe (DMSO)	200 µl	Violet	BN01039-8
SAM Reconstitution Buffer	500 µl	Amber	BN01039-9

IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well fluorescence plate reader
- Multi-channel pipette
- Isopropyl Alcohol pre-chilled to -20°C
- Dimethylsulfoxide (DMSO)

V. Storage and Handling:

Store kit at -80°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VI. Reagent Preparation and Storage Conditions:

- **NNMT Assay Buffer:** Warm to 37°C before use.
- **NNMT Enzyme:** Aliquot after the first thaw and store at -80°C. Stable at -80°C for two months. Avoid repeated freeze/thaw. Keep on ice while in use.
- **S-Adenosylmethionine (SAM) (Lyophilized):** Reconstitute each vial with 55 µl SAM Reconstitution Buffer as needed. Pipette up and down to dissolve completely. Store at -80°C. Avoid repeated freeze/thaw. Use reconstituted SAM within two weeks. Keep on ice while in use. Lyophilized product is stable at -80°C for two months.
- **Nicotinamide:** Store at -80°C. Use within two months.
- **Enzyme-I:** Aliquot after the first thaw and store at -80°C. Stable at -80°C for two months. Avoid repeated freeze/thaw. Keep on ice while in use.
- **Enzyme-II (Lyophilized):** Reconstitute with 220 µl NNMT Assay Buffer. Aliquot and store at –80°C. Stable for two months at –80°C.
- **1-Methylnicotinamide (MNA):** Store at -80°C. Avoid repeated freeze/thaw. Use within two months.
- **Thiol Detecting Probe:** Store at -20°C or -80°C. Thaw and mix well before use.
- **SAM Reconstitution Buffer:** Store at-20°C or -80°C

VII. NNMT Inhibitor Screening Protocol:

1. **Screening Compounds, Inhibitor Control & Blank Control Preparation:** Dissolve test inhibitors in an appropriate solvent to make a 3-100X stock solution. Dilute to 3X the highest desired test concentration with NNMT Assay Buffer. Prepare the reactions as shown in the table. If desired, serial dilutions of test inhibitors may be performed at this time, to a final volume of 50 µl.

	Sample	Enzyme Control	Background Control*	Inhibitor Control
Test Inhibitor (3X)	50 µl	–	–	–
NNMT Assay Buffer	–	50 µl	75 µl	48 µl
Inhibitor Control (MNA)	–	–	–	2 µl

Note:
(a) If desired, include a Solvent Control to test the effect of the solvent on enzyme activity. NNMT is sensitive to as low as 0.2 % DMSO in the assay.
(b) * The Thiol Detecting Probe will react with thiol groups in the enzymes used in the assay, hence a Background Control (BC) containing the reaction mix only without any Nicotinamide is necessary.

2. **NNMT Reaction Mix:** Dilute NNMT Enzyme and Enzyme-I 1:5 in NNMT Assay Buffer. Prepare a 75 µl Reaction Mix for each well (Sample, Enzyme Control, Background Control and Inhibitor Control) as follows.

NNMT Assay Buffer	58.5 µl
1:5 diluted NNMT Enzyme	2.5 µl
1:5 diluted Enzyme-I	10 µl
SAM	2 µl
Enzyme II	2 µl

Mix and add 75 µl/well. Mix well**.
** **Note:** Mix the contents in the wells thoroughly using a multichannel pipette.

3. **NNMT Assay:** To all wells, except the Background Control, add 25 µl Nicotinamide using a multi-channel pipette. Mix well** and incubate at 37ºC for 15 minutes. Stop the reaction by adding 50 µl of pre-chilled isopropyl alcohol (not provided) into each well, mix** and keep on ice for 5 minutes. For each well, prepare 50 µl of Thiol Detecting Probe working solution by adding 2 µl Thiol Detecting Probe into 48 µl DMSO (not provided) just before use. Add 50 µl of Thiol Detecting Probe working solution into each well. Mix** and incubate at room temperature for 5 minutes and read immediately.

   Note: Follow protocol exactly as described. Any deviations can result in sub-optimal results.

4. **Measurement:** Measure fluorescence (Ex/Em = 392/482 nm).

5. **Calculation:** Subtract the Background Control reading from all (Sample, Enzyme Control and Inhibitor Control) readings to obtain ΔRFU for each. Set the ΔRFU of Enzyme Control [EC] as 100%, and calculate % Inhibition or % Relative Activity of the test inhibitors as follows:

$$\% \text{ Inhibition} = \frac{\Delta\text{RFU of EC} - \Delta\text{RFU of S}}{\Delta\text{RFU of EC}} \times 100$$

$$\% \text{ Relative Activity} = \frac{\Delta\text{RFU of S}}{\Delta\text{RFU of EC}} \times 100$$

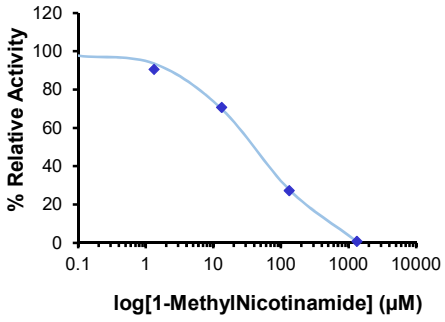


Figure: Inhibition of NNMT activity by 1-Methylnicotinamide. Assays were performed following the kit protocol.

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