Ab284513 – N'-Nicotinamide Methyltransferase (NNMT) Inhibitor Screening Assay Kit (Fluorometric)

For the screening of potential NNMT inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284513

Storage and Stability

On receipt entire assay kit should be stored at -80°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
1-Methylnicotinamide (150 mM)	20 µl	-80°C
Enzyme-I	200 µl	-80°C
Enzyme-II	1 Vial	-80°C
Nicotinamide	3 x 1.5 mL	-80°C
NNMT Assay Buffer	22 mL	-80°C
NNMT Enzyme	50 µl	-80°C
S-Adenosylmethionine	4 vials	-80°C
SAM Reconstitution Buffer	500 µl	-80°C
Thiol Detecting Probe (DMSO)	200 µl	-80°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

1-Methylnicotinamide (MNA): Store at -80°C. Avoid repeated freeze/thaw. 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

Nicotinamide: Store at -80°C. Use within two months.

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.

SAM Reconstitution Buffer: Store at-20°C or -80°C

Thiol Detecting Probe: Store at -20°C or -80°C. Thaw and mix well before use.

Assay Protocol

Screening Compounds, Inhibitor Control & Blank Control Preparation:

1. Dissolve test inhibitors in an appropriate solvent to make a 3-100X stock solution.
2. Dilute to 3X the highest desired test concentration with NNMT Assay Buffer.
3. 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

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.
* 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.

NNMT Reaction Mix:

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

	Reaction Mix
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

3. Mix and add 75 µl/well. Mix well**.

** Note: Mix the contents in the wells thoroughly using a multichannel pipette.

NNMT Assay:

1. To all wells, except the Background Control, add 25 µl Nicotinamide using a multi-channel pipette. Mix well** and incubate at 37°C for 15 minutes.
2. 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.
3. 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.

4. 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.

Measurement

Measure fluorescence (Ex/Em = 392/482 nm).

Calculation:

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

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

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

Technical Support

For all technical or commercial enquiries please go to:

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