ab308276 – NNMT inhibitor screening kit (Fluorometric)

For screening, studying and characterizing potential inhibitors of NNMT.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

Store kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
NNMT Assay Buffer (Avoid light)	25 ml	4°C or -20°C
DTT (1 M)	100 µl	-20°C
NNMT Enzyme	50 µl	-20°C
S-Adenosylmethionine (Lyophilized) (Avoid light)	1 vial	-20°C
NNMT Substrate	35 µl	-20°C
1-Methylnicotinamide	20 µl	-20°C

Materials Required, Not Supplied

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

- 96-well white plate with flat bottom
- Multi-well fluorescence plate reader
- dH_2O

Reagent Preparation

NNMT Assay Buffer: Store at 4 °C or -20 °C. Bring to room temperature (RT) before use.

DTT (1 M): Store at -20 °C. Prepare NNMT Assay Buffer containing 1 mM DTT immediately before use. Add 2 µl of DTT (1 M) stock solution to 1998 µl of NNMT Assay Buffer.

NNMT Enzyme: Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Stable for two months.

S-Adenosylmethionine (SAM): Reconstitute the vial in 40 µl dH_2O. Pipette up and down to dissolve completely. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within two months.

NNMT Substrate: Store at -20 °C, protected from light. Keep on ice while in use. Use within two months. **Note:** NNMT Substrate has a strong odour. Wear gloves and mask when handling.

1-Methylnicotinamide (MNA): Store at -20 °C. Keep on ice while in use. Use within two months.

NNMT Inhibitor Screening Protocol:

1. Screening Compounds, Inhibitor Control & Blank Control Preparation:

Δ Note: *Prepare NNMT Assay Buffer containing 1 mM DTT immediately before the assay.*

Sample Compound: Dissolve Test sample(s) in an appropriate solvent to make stock solution (see VI. 1. Note). Further dilute to 10 X using NNMT Assay Buffer containing 1 mM DTT. Add 10 µl of Diluted test sample(s) into wells of 96-well white plate designated as Sample [S].

Enzyme Control (No Inhibitor) and Background Control: Add 10 µl of NNMT Assay Buffer containing 1 mM DTT into designed well(s) of 96-well white plate designated as Enzyme Control [EC] and Background Control [BC] respectively.

Inhibitor Control (1-Methylnicotinamide; MNA): Prepare a 10-fold dilution of MNA by adding 2 µl of the MNA stock solution to 18 µl of NNMT Assay Buffer containing 1 mM DTT, mix well. Add 10 µl of diluted MNA into designated well(s) of 96-well white plate designated as Inhibitor Control [IC].

Δ Note: *Various organic solvents may reduce the NNMT enzymatic activity (see Fig b). Prepare the stock samples in dH_2O, if possible. If samples have to be prepared in an organic solvent, we recommend dissolving the test samples to 1000X or higher concentrations. Further dilute the test samples(s) with NNMT Assay Buffer containing 1 mM DTT to minimize the effect of organic solvent(s). Prepare parallel well(s) with the same final concentration of organic solvent as Solvent Control [SC] to test the effect of the solvent on NNMT activity. It is strongly recommended that if you use any organic solvent for preparing the test inhibitors you have to use the Solvent Control well signal instead of the Enzyme Control well signal.*

	[S]	[IC]	[EC]	[BC]	[SC]
Test Sample	10 µl	-	-	-	-
Diluted MNA	-	10 µl	-	-	-
Assay Buffer containing 1 mM DTT	-	-	10 µl	10 µl	-
Solvent Control	-	-	-	-	10 µl

NNMT Enzyme Preparation:

Prepare 60-fold dilution of NNMT Enzyme in NNMT Assay Buffer containing 1 mM DTT. For example, add 2 µl of NNMT Enzyme with 118 µl NNMT Assay Buffer /1 mM DTT), mix well. Mix enough reagents for the number of assays to be performed.

	Enzyme Mix	Background Mix
Diluted NNMT	30 µl	---
NNMT Assay Buffer containing 1 mM DTT	40 µl	70 µl

Add 70 µl of Enzyme Mix to test sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC] and Solvent Control [SC] wells and mix well. Add 70 µl of Background Mix to the Background Control [BC] well. Incubate the plate at 37 °C for 15~20 min, protected from light.

NNMT Reaction Mix:

Prepare 50-fold dilution of SAM. For example, dilute 2 µl of SAM stock solution with 98 µl NNMT Assay Buffer containing 1 mM DTT. Prepare 50-fold dilution of NNMT substrate. For example, dilute 2 µl of NNMT substrate stock solution with 98 µl NNMT Assay Buffer containing 1 mM DTT. Mix enough reagents for the number of assays to be performed. For each well, prepare a total 20 µl NNMT Reaction Mix as follows:

	NNMT Reaction Mix
Diluted NNMT Substrate	10 µl

Diluted SAM	10 µl

Mix well and add 20 µl NNMT Reaction to all wells including test sample(s), Inhibitor Control, [EC], [SC] and [BC] wells. Mix well. *The total final reaction volume for each well will be 100 µl.*

Δ Note: *Prepare NNMT Reaction Mix immediately before adding to the wells.*

Measurement:

Immediately measure the fluorescence (Ex/Em = 320/420 nm) in kinetic mode at 37 °C for 30~60 min using a fluorometric microtiter plate reader. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding RFU for Sample (R_{S1} and R_{S2}) and Background Control ($R_{B1}$and R_{B2}).

Δ Note: The Enzyme progressive curve is hyperbolic, with an initial linear portion followed by progressively slower reaction. Use the initial portion to check the linear range of the reaction.

Calculation:

Calculate the slope for all test samples [S], Enzyme Control [EC], Solvent Control [SC] and Background Controls [BC] by dividing the net ΔRFU (R_{t2}-R_{t1}) values over reaction time Δt (t_2-t_1). Subtract the Slope of Background Control values from [S], [EC] and [SC]. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine effect of tested compound.

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

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

Technical Support

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