Tribioscience

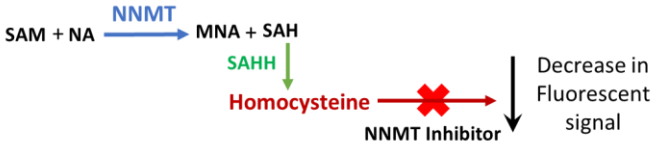
NNMT Inhibitor Screening Assay (Catalog: TBS2097, 100 Assays, Store at -20 ℃)

DESCRIPTION

NNMT (Nicotinamide N-Methyltransferase) is an enzyme that catalyzes the methylation of nicotinamide and other pyridines using S-adenosyl-L-methionine (SAM) as the methyl group donor to produce SAH and 1-methylnicotinamide. NNMT plays a significant role in the regulation of metabolic pathways. It is expressed at high levels in several kinds of cancers, neurodegenerative diseases, obesity, and diabetes, indicating a potential target for therapy.

Tribioscience's NNMT inhibitor screening kit is a fluorometric assay that utilizes SAM and nicotinamide as the substrates to generate SAH which is hydrolyzed to form homocysteine. The free thiol group of the homocysteine can be detected with a thiol probe generating an enhanced fluorescent signal at Ex/Em = 400/465 nm. In the presence of an NNMT inhibitor, the fluorescent signal is reduced in concentration dependent manner. The kit provides the easiest, most accurate, and simplest approach for high-throughput screening of NNMT inhibitors.

Fig. 1: Principle of NNMT Inhibitor Screening Assay



APPLICATIONS

NNMT inhibitor screening assay.

KIT CONTENTS FOR 100 TESTS:

Name	Size (100 tests)
NNMT Substrate SAM	60 µL
NNMT Substrate NA	40 µL
NNMT Assay Buffer	12 mL
Thiol Detecting Probe (80X)	80 µL
Enzyme mix II	2500 µL
Enzyme I	40 µL
1-Methylnicotinamide (MNA, 100 mM)	50 µL

Storage conditions: Store the Reagent at –20°C protected from light. Shelf life: 12 months. For Enzyme I and Enzyme II, long term storage at –80°C.

PROCEDURES

1. Equilibrate all the kit components until room temperature before starting the experiment. Use a black microplate.
2. Prepare the screening compound, inhibitor, and blank control: Dissolve the test compounds in an appropriate solvent, further diluting the compound in the NNMT assay buffer. The effect of the solvent on the NNMT activity should be considered by including a solvent control to the assay. Add 10 µL of the test compound and the diluted MNA to a black microplate. Add the components as shown in Table 1.

Table.1: Assay preparation

	Test Inhibitor	Inhibitor Control	Enzyme Control	Blank Control	Solvent Control
Test Inhibitor	10 µL				
Diluted MNA		10 µL			
Assay Buffer			10 µL	22.5 µL	
Solvent Control					10 µL

3. Add 10 µL the Test compound, Inhibitor Control and Solvent Control to the wells, add 10 µL of assay buffer to the Enzyme control, and 22.5 µL of assay buffer to the blank control.
4. Prepare Enzyme I working solution: Add 25 µL of the Enzyme I to 1225 µL of the NNMT assay buffer, for 100 wells. Please adjust the volume as needed (Note: The diluted Enzyme I working solution is fresh use only, cannot be stored for future use). Then add 12.5 µL to the wells, except the blank control.
5. Prepare the substrate Mix: Add 1208 µL assay buffer, 30 µL NNMT Substrate SAM, 12 µL Substrate NA, and mix well. The total volume of 1250 µL substrate mix is enough for 100 assays. Please adjust the volume as you need and store the unused portion of substrate at -20°C.
6. Add 12.5 µL of substrate mix to the wells.
7. Incubate at 37°C for 30 minutes with gentle shaking and protected from light.
8. Add 25 µL of Enzyme mix II, gently tap the plate to mix well.
9. Incubate the microplate at 37°C for 1 hour with gentle shaking and protected from light.
10. Preparation of the thiol detection probe working solution: add 50 µL of Thiol Detecting Probe (80X) to 3950 µL of DMSO.
11. Add 40 µL of the thiol detection working solution to the wells.
12. Incubate at 37°C for 10 minutes with gentle shaking and protected from light.
13. Read the plate at excitation and emission wavelength at Ex/Em = 400 nm/465 nm respectively.
14. Calculation:
    Subtract the Blank Control reading from all readings (Compound, Enzyme Control, and Inhibitor Control). Set the ΔRFU of Enzyme Control (EC) as 100%.

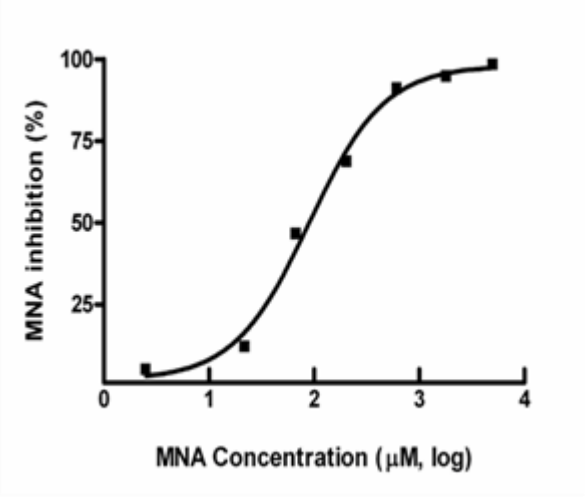
% of Inhibition = 100* [ΔRFU(EC)- ΔRFU(TC)] /ΔRFU(EC)

Here EC=Enzyme Control; TC= Test Compound.

15. typical data displays in Fig.2.

2011 © by Tribioscience **365 San Aleso Ave, Sunnyvale, CA 94085, USA** · Website: www.tribioscience.com
Tel: 408-498-0197/ 650-917-9269, Fax: 650-618-5498· Email: order@tribioscience.com

Fig. 2. Inhibition of NNMT activity by MNA



RELATIVE PRODUCTS

Resazurin Cell Viability Kit (TBS2001)
CCK-8 Cell Viability Assay (TBS2022)
GOT Activity Assay (TBS2013)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Homocysteine Fluorometric Assay (TBS2091)
NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
Caspase-3 Fluorometric Assay kit (TBS2030)
Caspase-1 Fluorometric Assay (TBS2045)

This product is for research use only.