Tribioscience

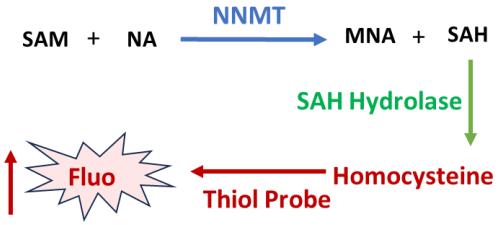
NNMT Activity Fluorometric Assay (Catalog: TBS2098, 100 Assays, Store at -20 ℃)

DESCRIPTION

NNMT (Nicotinamide N-Methyltransferase) is an enzyme that catalyzes the methylation of nicotinamide (NA) and other pyridines using S-adenosyl-L-methionine (SAM) as the methyl group donor to produce SAH and 1-methylnicotinamide (MNA). 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 Activity Assay 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. The assay principle is displayed in Figure 1. The kit provides the easiest and most accurate approach to measure NNMT activity in a variety of samples.

Fig. 1: Principle of NNMT Assay



APPLICATIONS

Measure NNMT activity in a variety of samples.

KIT CONTENTS FOR 100 TESTS:

Name	Size (100 tests)
NNMT Substrate SAM	60 µL
NNMT Substrate NA	30 µL
NNMT Assay Buffer	20 mL
Thiol Probe (100X)	60 µL
SAH Enzyme Mix	2500 µL
NNMT positive control	50 µL
GSH stock (400 µM)	80 µL

Storage conditions: Store the Reagent at –20° (–80°C for enzyme NNMT and SAH Enzyme Mix), protected from light. Shelf life: 6 months.

PROCEDURES

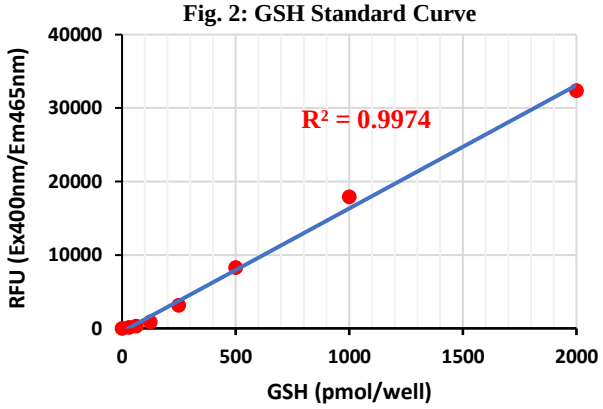
1. Equilibrate all the kit components until room temperature before starting the experiment.
2. Prepare the GSH standard: Add 30 µL of GSH to 270 µL of assay buffer, and then do a 2-fold serial dilution as the Table1.

Table 1: Standard Preparation

Tube#	Vol. (µL) GSH Standard	Vol. (µL) Assay Buffer	GSH concentration (µM)	GSH (pmol/Well)
1	30	270	40	2000
2	150	150	20	1000
3	150	150	10	500
4	150	150	5	250
5	150	150	2.5	125
6	150	150	1.25	62.5
7	150	150	0.625	31.25
8	0	150	0	0

3. Prepare the substrate Mix: Add assay buffer 1208 µL, NNMT Substrate SAM 30 µL and Substrate NA 12 µL, mix well, total volume 1250 µL. This is for 100 assays, please adjust the volume as you need and store the unused portion of substrate at -20℃.
4. Preparation of the working solution for Thiol Detecting Probe: add 50 µL of Thiol Detecting Probe (100X) to 4950 µL of DMSO.
5. Add 12.5 µL of substrate mix to the wells of a black microplate.
6. Add 12.5 µL of sample, or the NNMT positive control to the indicated wells in duplicate manner.
7. Incubate at 37℃ for 30 minutes with gentle shaking and protected from light.
8. Add 25 µL of SAH Enzyme Mix, gently tap to mix.
9. Incubate the microplate at 37℃ for 1 hour with gentle shaking and protected from light.
10. Add 50 µL of GSH standards to indicated wells in duplicate manner.
11. Add 50 µL of the thiol detection working solution to the test samples, GSH standards, and NNMT positive control.
12. Incubate at 37℃ 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. The typical GSH standard curve is shown in Fig. 2.

Fig. 2: GSH Standard Curve



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16. Calculate the NNMT activity by the typical GSH standard curve as follows:

Y=A*X + B

The free thiol product (pmol): X = (Y -B)/ A

The NNMT Activity (pmol/min/μg):
= **(X * DF) / (T * S)**

Here: **Y= RFU; A=Slope; B= constant value; X= Free Thiol product (pmol); DF=dilution factor; T=incubate Time (min); S=sample amount (μg)**

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 Inhibitor Screening Assay (TBS2097)
G6PDH Activity Colorimetric Assay (TBS2102)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
Caspase-3 Colorimetric Assay kit (TBS2030)

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