Description

The NMT2 Fluorogenic Assay Kit is designed to measure NMT2 (N-myristoyltransferase 2) N-myristoylation activity for screening and profiling applications. The kit comes in a convenient 96-well format, with purified recombinant NMT2 (amino acids 112-498), NMT Peptide 1, Myristoyl – coenzyme A (MCoA), CPM (fluorescent dye), and assay buffer for 100 enzyme reactions.

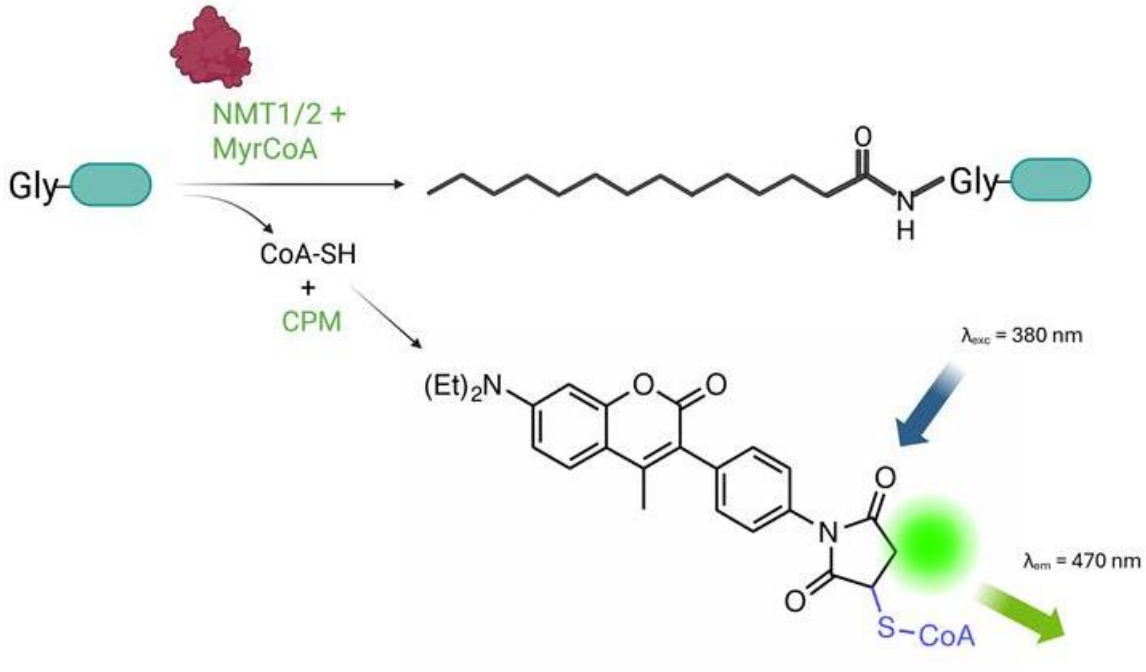

Figure 1. Mechanism of action of NMT2 Fluorogenic Assay Kit.

Background

NMT2 (N-myristoyltransferase 2), also known as Glycylpeptide N-tetradecanoyltransferase 2, is a member of the NMT family of proteins. It is a cytosolic enzyme that catalyzes the addition of a myristoyl group to glycine residues located in the N-terminus of eukaryotic, fungal and viral proteins. The addition of the myristoyl group allows for proper protein localization, and for cell survival and proliferation. Several cancer types present up-regulation of this protein, for example colorectal cancer. It has also been linked to cardiovascular disorders, being found at low levels in damaged hearts. Studies have indicated that inhibition of NMT can be a promising therapeutic approach in oncology. Further studies into the role of this protein will open new treatment opportunities in cancer therapy.

Applications

Screen small molecule inhibitors or antagonists that affect activity of NMT2 in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102924-KC10	NMT2, His-tag*	10 µg	-80°C
84139-KC5	NMT Peptide 1 (5 mM)	5 µl	-80°C
84140-KC5	Myristoyl – coenzyme A (MCoA) (5 mM)	5 µl	-80°C
84141-KC5	CPM (20 mM)	5 µl	-80°C
84142-KC10	1x NMT Assay Buffer	2 x 10 ml	-20°C
79685	96-well black, low binding microplate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Fluorescent microplate reader capable of reading λexc/λem= 380 nm/470 nm
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the reaction should not exceed 3%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound with the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Zelenirstat (#84233) as internal controls for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw **1x NMT Assay Buffer** and **NMT2**. Briefly spin the tube to recover its full content.

2. Add 30 µl of 1x NMT Assay Buffer to the "Blank" wells.

3. Dilute **NMT2** to 3.33 ng/µl (30 µl/well) with 1x NMT Assay Buffer.

4. Add 30 µl of diluted NMT2 to the "Positive Control" and "Test Inhibitor" wells.

5. Prepare the **Test inhibitor** (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x NMT Assay Buffer at concentrations 10-fold higher than the desired final concentrations.

   For positive and negative controls, use 1x NMT Assay Buffer (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in 1x NMT Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using 1x NMT Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in 1x NMT Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO coming from the test inhibitor should not exceed 1%.

6. Add 5 µl of **Test inhibitor** to each well designated "Test Inhibitor".

7. Add 5 µl of **Diluent Solution** to the "Blank" and "Positive Control" wells.

8. Preincubate the plate for 60 minutes at Room Temperature (RT) with gentle agitation.

9. Thaw **NMT Peptide 1**, **Myristoyl – coenzyme A (MCoA)**, and **CPM**. Briefly spin the tubes to recover their full content.

10. Dilute 100-fold the **NMT Peptide 1** with 1x NMT Assay Buffer (5 µl/ well), to 50 µM.

11. Dilute **CPM** 400-fold with 1x NMT Assay Buffer (5 µl/ well), to 50 µM.

12. Dilute **MCoA** 100-fold with 1x NMT Assay Buffer (5 µl/ well), to 50 µM.

13. Prepare a **Master Mix** (15 µl/well): N wells x (5 µl of diluted NMT Peptide 1 + 5 µl of diluted CPM + 5 µl of diluted MCoA).

    Note: Prepare Master Mix in excess (usually 10-20% extra) to account for pipetting losses, spills, or errors.

14. Add 15 µl of Master Mix to all wells to initiate the reaction.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
1x NMT Assay Buffer	30 µl	-	-
Diluted NMT2 (3.33 ng/µl)	-	30 µl	30 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Pre-incubate 60 minutes at RT			
Master Mix	15 µl	15 µl	15 µl
Reaction	**50 µl**	**50 µl**	**50 µl**

15. Incubate at RT for 60 minutes or perform kinetic analysis.

16. Read the fluorescence intensity of the samples (λexcitation = 380 nm; λemission = 470 nm with a bandwidth of 20 nm) in an appropriate microplate reader.

17. The "Blank" value should be subtracted from all other values.

Example Results

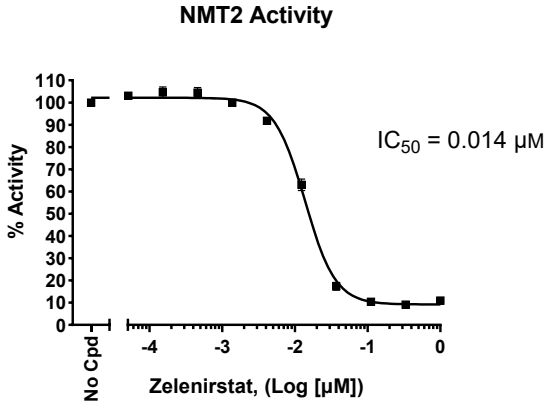

Figure 2. Inhibition of NMT2 activity by Zelenirstat.
NMT2 activity was measured in the presence of increasing concentrations of Zelenirstat. Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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