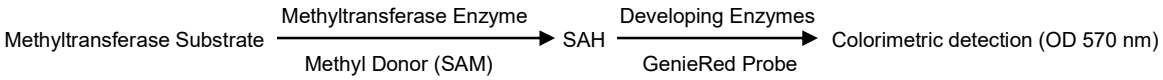
Methyltransferase Activity Assay Kit (Colorimetric) (#BN01144)

(Catalog # BN01144; 100 assays; Store at -80°C)

I. Introduction:

Methyltransferases are a diverse group of enzymes that methylate proteins, DNA, and small molecule targets. Accordingly, methyl transferase activity plays a critical role in modulating gene transcription, protein interactions, and signaling networks. Anomalous DNA methylation patterns have been consistently detected during cancer development as well as in many genetic disorders such as Fragile X Syndrome, ICF, and Rett Syndrome. Assay Genie's Methyltransferase Activity Assay Kit (Colorimetric) allows for kinetic evaluation of methyltransferase activity of purified enzymes and their inhibitors. The transfer of a methyl group from S-Adenosyl Methionine (SAM) cofactor to a corresponding substrate generates S-Adenosyl Homocysteine (SAH) as a product. SAH is detected by coupling the methyl transfer reaction to a multi-step enzymatic cascade, resulting in the generation of an intermediate that reacts with GenieRed Probe. The reaction product exhibits a strong absorbance at 570 nm. The limit of quantification (L.O.Q) is 296 pmol of SAH generated per min per ml (296 µU/ml) of purified enzymes.



II. Applications:

- Measurement of methyltransferase activity of purified proteins
- Analysis of methyltransferase inhibitors

III. Sample Type:

- Purified Protein

IV. Kit Contents:

Components	BN01144	Cap Code	Part Number
MT Assay Buffer	25 ml	WM	BN01144-1
Enzyme Re-Suspension Buffer	1 ml	White	BN01144-2
Enzyme Mix I	1 vial	Purple	BN01144-3
Enzyme Mix II	1 vial	Clear	BN01144-4
Enzyme Mix III	3 X 200 µl	Blue	BN01144-5
SAM Cofactor (50 mM)	250 µl	Orange	BN01144-6
SAH Standard (50 mM)	100 µl	Brown/Amber	BN01144-7
MT Positive Control	65 µl	Green	BN01144-8
GenieRed Probe	200 µl	Red/Amber	BN01144-9

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Microplate reader
- Purified Methyltransferase (i.e. NNMT) and its corresponding Substrate (i.e. Nicotinamide) pair

VI. Storage Conditions and Reagent Preparation:

Store kit at -80°C, protected from light. Briefly centrifuge small vials prior to opening. Although multiple freeze-thaw cycles are not recommended, re-freeze unused assay components in liquid nitrogen prior to storage at -80°C if necessary. Read entire protocol before performing the assay.

- **MT Assay Buffer:** Store at 4 ºC. Warm to 37 ºC temperature before use.
- **Enzyme Re-Suspension Buffer:** Ready to use. Store at -20ºC.
- **Enzyme Mix I and Enzyme Mix II:** Reconstitute each mix with 210 µl of **Enzyme Re-Suspension Buffer**. Gently pipette up and down to dissolve completely and centrifuge for 1 min at 4°C on max to remove any foaming that may have occurred. Aliquot out upon initial use and avoid freeze-thawing more than twice. Thaw enzyme mix solutions on ice before use. Store at -80°C.
- **Enzyme Mix III and SAM Cofactor (50 mM):** Ready to use. Thaw on ice and aliquot out upon initial use. Avoid freeze-thawing more than twice. Store at -80°C.
- **SAH Standard (50 mM) and GenieRed Probe:** Store at -80°C. Make sure the standard and the probe are completely thawed at room temperature prior to use. Aliquot upon initial use to avoid freeze-thaw cycles and protect from light.
- **MT Positive Control:** Thaw on ice and briefly centrifuge. Store at -80°C.

VII. Methyltransferase Activity Assay Protocol:

1. **Sample Preparation:** Thaw purified Methyltransferase and its corresponding Substrate along with all the provided assay components on ice, unless otherwise stated. Dilute Methyltransferase and its Substrate to a desired concentration with **MT Assay Buffer**. Combine a desired amount of Methyltransferase and its Substrate and adjust the volume to 50 µl with MT Assay Buffer in a 96-well clear plate. Use buffer only (no Methyltransferase) for background control reaction. For positive control reaction, mix 6 µl of MT Positive Control with 44 µl of MT Assay Buffer.

   Notes:

   a. Do not store enzyme/substrate diluted in MT Assay Buffer; discard the dilutions after use.

   b. For uncharacterized enzymes, we suggest testing several doses to ensure the reading is within the Standard Curve range.

2. **SAH Standard Curve:** Prepare 200 µM SAH Standard stock in MT Assay Buffer by diluting 4 µl of 50 mM SAH Standard in 996 µl MT Assay Buffer. Add 0 (Background Control), 5, 15, 25, 35, 45 µl of 200 µM SAH standard into a series of wells on a 96-well plate to generate 0, 1, 3, 5, 7, 9 nmol/well of SAH Standard. Adjust the volume to 50 µl with MT Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of samples and standards to be performed:

	Sample Reaction Mix (1 assay)
MT Assay Buffer	37 µl
Enzyme Mix I	2 µl
Enzyme Mix II	2 µl
Enzyme Mix III	6 µl
SAM Cofactor (50 mM)	1 µl
GenieRed Probe	2 µl

Mix and add 50 µl of the Sample Reaction Mix to each well containing the Positive Control, Test Samples, Standards and Background Control.

4. **Measurement:** For positive control, test samples, and background control measure absorbance at 570 nm in kinetic mode every 30 seconds for at least 45 minutes at 37 °C. To generate the SAH Standard Curve, incubate SAH standard reactions for 45 minutes at 37 °C and measure absorbance at 570 nm in end-point mode or simply take absorbance reading at the 45 minute mark from the kinetic reading.

Note: Your sample methyltransferase may have a different optimal temperature. You may change reaction temperature to suit your needs. Similarly, your sample methyltransferase may have a different K_M for the SAM Cofactor. In this case, the user may decide on the optimal SAM Cofactor concentration to incorporate into the master mix. For Methyltransferase enzymes showing very low activities, it may be advantageous to use a more sensitive Methyltransferase Activity Assay Kit (Fluorometric).

5. **Calculations:** *Standard Curve:* Subtract 0 nmol SAH Standard reading from all SAH standards to obtain normalized standard curve. Plot the SAH standard curve. Apply a linear fit to the SAH standard values and determine the standard curve equation. *Samples/Positive Control:* subtract each point on the "no methyltransferase" background control curve from each corresponding point generated in Sample and Positive Control readings. Apply OD values at each time point to the SAH standard curve equation to determine nmol of SAH generated at each time point; multiply these values by 1000 to determine pmol of SAH generated at each time point. Plot pmol SAH on the y-axis vs. time (in minutes) on the x-axis and determine the sample reaction slope slope (pmol/min) of the linear portion of the curve.

Sample Methyltransferase Activity = [sample reaction slope/V] x D (pmol/min/ml ≡ µU/ml)

Sample Specific Activity = slope/µg (pmol/min/µg ≡ µU/µg)

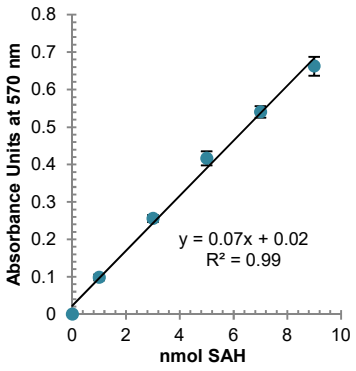
Where: **V =** sample volume added into the reaction well (ml).

D = Dilution Factor

Sample Reaction Slope = pmol/min (calculated using the SAH standard curve equation)

Unit Definition: One unit of methyltransferase is the amount of enzyme that generates 1.0 µmol of SAH per min. at 37°C.

a)



b)

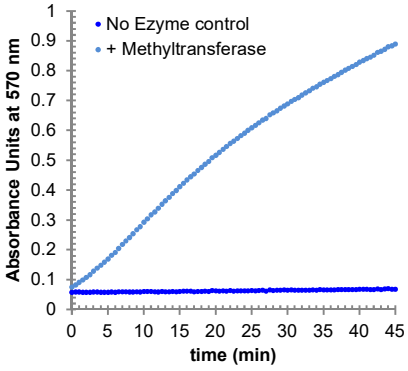


Figure: a) Normalized SAH Standard Curve; **b)** Representative activity curve for human recombinant Nicotinamide N-Methyltransferase (NNMT) with Nicotinamide Substrate at 37°C. Assays were performed using kit protocol.

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