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Methyltransferase Activity Assay Kit (Fluorometric)

07/16

(Catalog # K521-100; 100 assays; Store at -80°C)

I. Introduction:

Methyltransferases are a diverse group of enzymes that function to methylate protein, 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 pattern has been consistently detected during cancer development as well as in many genetic disorders such as Fragile X Syndrome, ICF, and Rett Syndrome. BioVision's Methyltransferase Activity Fluorimetric Assay Kit allows for kinetic evaluation of methyltransferase activity of purified enzymes. The transfer of a methyl group from S-Adenosyl Methionine (SAM) cofactor to a corresponding substrate generates S-Adenosyl Homocysteine (SAH) 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 OxiRed™ Probe. The fluorescent product is measured at Ex/Em = 535/587 nm. The limit of quantification (L.O.Q) is 7.5 pmol of SAH generated per min per ml (7.5 µU/ml) in purified enzymes.

Methyltransferase Substrate —(Methyltransferase Enzyme / Methyl Donor (SAM))→ SAH —(Developing Enzymes / OxiRed™ Probe)→ Fluorometric detection (Ex/Em = 535/587 nm)

II. Applications:

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

III. Sample Type:

- Purified Protein

IV. Kit Contents:

Components	K521-100	Cap Code	Part Number
MT Assay Buffer	25 ml	WM	K521-100-1
Enzyme Re-Suspension Buffer	1 ml	White	K521-100-2
Enzyme Mix I	1 vial	Blue	K521-100-3
Enzyme Mix II	1 vial	Clear	K521-100-4
Enzyme Mix III	200 µl	Brown	K521-100-5
SAM Cofactor (0.5 mM)	500 µl	Orange	K521-100-6
SAH Standard (100 µM)	500 µl	Yellow/Amber	K521-100-7
MT Positive Control	25 µl	Green	K521-100-8
OxiRed™ Probe	200 µl	Red/Amber	K521-100-9

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom, low-medium binding
- Fluorometer
- Purified Methyltransferase (i.e. PRMT1) and its corresponding Substrate (i.e. purified histone H4) 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 25 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 (0.5 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 (100 µM) and OxiRed™ 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. If any precipitation occurs, re-suspend the solution by gently pipetting up and down several times. 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. Alternatively, if Substrate concentration is to be the same across all samples, incorporate the Substrate into the reaction master mix in step 2. Use buffer only (or no Methyltransferase) for background control reaction. For positive control reaction, add 4 µl of MT Positive Control in 46 µl of MT Assay Buffer.

Notes:

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

b. If your sample reading at t=0 is significantly higher than background control, treat you sample with catalase beads (BV Cat# 7931) prior to use.

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

2. **SAH Standard Curve:** Prepare 2.5 µM SAH Standard stock in MT Assay Buffer by diluting 25 µl of 100 µM SAH Standard in 975 µl MT Assay Buffer. Add 0, 2, 6, 12, 18, 24 µl of 2.5 µM SAH standard into a series of wells on a 96-well plate to generate 0, 5, 15, 30, 45, 60 pmol/well of SAH Standard. Adjust the volume to 50 µl with MT Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well containing sample(s), and standards prepare 50 µl Mix containing:

	Sample Reaction Mix (1 assay)	SAH Standard Reaction Mix (7 assays)
MT Assay Buffer	up to 50 µl	287 µl
Enzyme Mix I	2 µl	14 µl
Enzyme Mix II	2 µl	14 µl
Enzyme Mix III	2 µl	14 µl
SAM Cofactor (0.5 mM)	2 µl	14 µl
Substrate	varying	----
OxiRed™ Probe	1 µl	7 µl

Mix and add 50 µl of the Reaction Mix to each well containing the Positive Control, test samples, and background control. Add 50 µl of SAH Standard Reaction Mix to each well containing SAH Standard.

4. **Measurement:** For positive control, test samples, and background control measure fluorescence (Ex/Em = 535/587 nm) in kinetic mode every 30 seconds for at least 30 minutes at 25 °C. Set-up the fluorometer to a low GAIN/PMT setting and medium to high setting for SENSITIVITY. To generate the SAH Standard Curve, incubate SAH standard reactions for 15 minutes at 25 °C and measure fluorescence at Ex/Em = 535/587 in End-point mode.

Note: Your sample methyltransferase may have a different optimal temperature. You may change reaction temperature to suit your needs, however, higher reaction temperature may result in higher background fluorescence. Similarly, your sample methyltransferase may have a different K_M for the SAM Cofactor. In this case, the user must decide on the optimal SAM Cofactor concentration to incorporate into the master mix.

5. **Calculations:** *Standard Curve:* Subtract 0 pmol SAH Standard reading from 0, 5, 15, 30, 45, 60 pmol of SAH standard to obtain normalized standard curve. Plot the SAH standard curve with pmol of SAH on the x-axis and normalized RFU on the y-axis. 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 curve from each corresponding point generated in Sample and Positive Control readings. Apply RFU values at each time point to the standard curve equation 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 slope (pmol/min) of the linear portion of the curve.

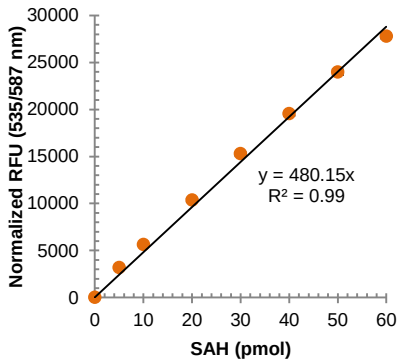
Sample Methyltransferase Activity = (slope/V) x D (pmol/min/ml ≡ µU/ml)

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

Where: **V =** sample volume added into the reaction well (ml).
D = Dilution Factor
Slope = pmol/min (from the SAH curve)

Unit Definition: One unit of methyltransferase is the amount of enzyme that generates 1.0 µmol of SAH per min. at 25°C.

a)



b)

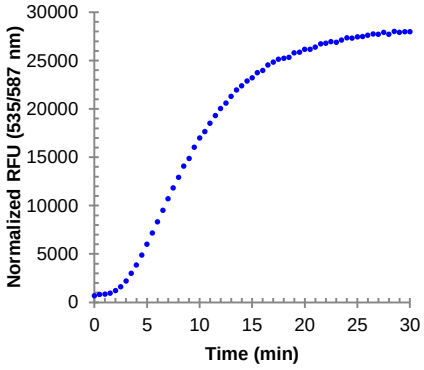


Figure: a) SAH Standard Curve; b) representative activity curve for human recombinant Protein Arginine Methyltransferase 1 (hPRMT1) (BV Cat No.4865) with Histone H4 Substrate (BV Cat. No. 7671) at 25°C. Assays were performed using kit protocol.

VIII. RELATED PRODUCTS:

Adenosylhomocysteinase (AHCY) Activity Fluorometric Assay Kit (K807)
Adenosine Deaminase Activity Assay Kit (Fluorometric) (K328)

FOR RESEARCH USE ONLY! Not to be used on humans.