Methyltransferase Activity Assay Kit (Fluorometric)

(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 transcription as well as in many genetic disorders such as Fragile X Syndrome, ICF, and Rett Syndrome. BioVision’s Methyltransferase Activity Fluorometric 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.

II. Applications:
- Measurement of methyltransferase activity of purified proteins
- Analysis of methyltransferase inhibitors

III. Sample Type:
- Purified Protein

IV. Kit Contents:

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

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 °C. Warm to 25 °C temperature before use.
- Enzyme Re-Suspension Buffer: Ready to use. Store at -20°C.
- 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 centrifugate 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.

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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, 0.5, 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:

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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.
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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. For positive control, test samples and background 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.

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Sample Methyltransferase Activity = (slope/V) x D (pmol/min/ml) = μU/ml
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Sample Specific Activity = (slope/μg) x D (pmol/min/μg) = μU/μg
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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 pmol of SAH per min. at 25°C.

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.**