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ab273307 Methyltransferase Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273307
(use https://www.abcam.cn/ab273307 for china, or
https://www.abcam.co.jp/ab273307 for Japan)

Kinetic evaluation of Methyltransferase activity of purified enzymes and their inhibitors.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Methyltransferase Activity Assay Kit (Colorimetric) (ab273307) 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 Red 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.

2. Protocol Summary

Prepare reagents following protocol.

Combine Methyltransferase and its Substrate and adjust to 50 µl in the well with MT Assay Buffer.

Prepare Negative and Positive Controls and Standards.

Prepare and add to the appropriate wells the Sample Reaction Mix.

Measure absorbance every 30 seconds in kinetic mode for at least 45 minutes at 37 °C.

Calculate Methyltransferase activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -80°C in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
MT Assay Buffer	25 mL	-20°C
Enzyme Re-Suspension Buffer	1 mL	-20°C
Enzyme Mix I (Lyophilized)	1 vial	-20°C
Enzyme Mix II (Lyophilized)	1 vial	-20°C
Enzyme Mix III	3 × 200 µL	-20°C
SAM Cofactor (50 mM)	250 µL	-20°C
SAH Standard (50 mM)	100 µL	-20°C
MT Positive Control	65 µL	-20°C
Red Probe	200 µL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 570 nm
- Purified Methyltransferase (i.e. NNMT) and its corresponding Substrate (i.e. Nicotinamide) pair
- 96-well clear plate with flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

- 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.

9.1 MT Assay Buffer:
Store at 4 °C and warm to 37 °C temperature before use.

9.2 Enzyme Re-Suspension Buffer:
Ready to use and store at -20°C.

9.3 Enzyme Mix I (Lyophilized) and Enzyme Mix II (Lyophilized):
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.

9.4 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.

9.5 SAH Standard (50 mM) and Red Probe:
Store at -80°C. Make sure the SAH Standard and the Red Probe are completely thawed at room temperature prior to use. Aliquot upon initial use to avoid freeze-thaw cycles and protect from light.

9.6 MT Positive Control:
Thaw on ice and briefly centrifuge. Store at -80°C.

10. Sample Preparation

10.1 Thaw purified Methyltransferase and its corresponding Substrate along with all the provided assay components on ice, unless otherwise stated.

10.2 Dilute Methyltransferase and its Substrate to a desired concentration with MT Assay Buffer.

10.3 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.

10.4 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.

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

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

11. Assay Procedure

Thaw all reagents thoroughly and mix gently.

SAH standard curve

11.1 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.

11.2 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.

11.3 Adjust the volume to 50 µl with MT Assay Buffer.

200 µM SAH Standard (µl)	MT Assay Buffer (µl)	SAH Standard (nmol/well)
45	5	9
35	15	7
25	25	5
15	35	3
5	45	1
0	80	0

Reaction Mix:

11.4 Mix enough reagents for the number of samples and standards to be performed. Table below shows volumes enough for one assay:

Components	Sample reaction (µl)
MT Assay Buffer	37
Enzyme Mix I	2
Enzyme Mix II	2
Enzyme Mix III	6
SAM Cofactor (50 mM)	1
Red Probe	2

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

Measurement:

11.6 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.

11.7 To generate the SAH Standard Curve, incubate SAH standard reactions for 45 minutes at 37 °C and measure absorbance at 570 nm or simply take absorbance reading at the 45 minutes mark from the kinetic reading.

Δ 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 KM 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 kit.

12. Calculations

12.1 Standard Curve:

12.1.1 Subtract 0 nmol SAH Standard reading from all SAH standards to obtain normalized standard curve.
12.1.2 Plot the SAH standard curve.
12.1.3 Apply a linear fit to the SAH standard values and determine the standard curve equation.

12.2 Samples/Positive Control:

12.2.1 Subtract each point on the no methyltransferase background control curve from each corresponding point generated in Sample and Positive Control readings.
12.2.2 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.
12.2.3 Plot pmol SAH on the y-axis vs. time (in minutes) on the x-axis and determine the sample reaction slope (pmol/min) of the linear portion of the curve.

$$Sample\ methyltransferase\ activity = \frac{sample\ reaction\ slope}{V}\ x\ D = \ \mu U/ml$$

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

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 minute at 37°C.

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

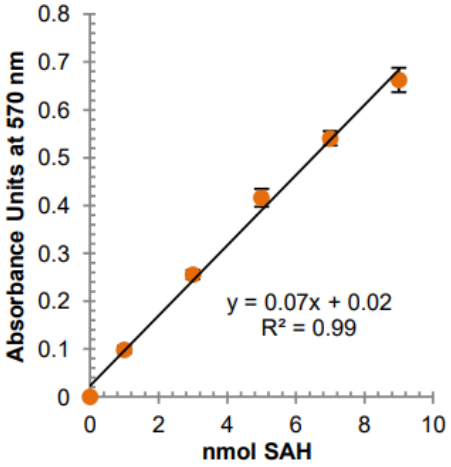

Figure 1. Typical SAH Standard Curve.

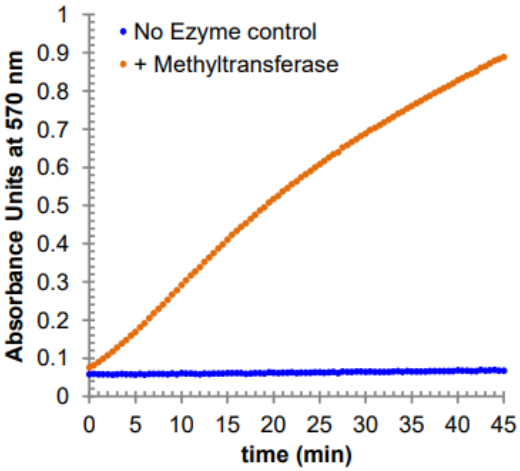

Figure 2. Representative activity curve for human recombinant Nicotinamide N-Methyltransferase (NNMT) with Nicotinamide Substrate.

14. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)