Methyltransferase Colorimetric Assay Kit

Item No. 700140

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GENERAL INFORMATION

Materials Supplied

This kit will arrive packaged as a -80°C kit. For best results, store the kit as supplied or remove components and store as stated below.

Item Number	Item	Quantity/Amount	Storage
700141	MT Assay Buffer	1 vial/20 ml	-20°C
700142	MT Assay Buffer Additive	1 vial/200 µl	-20°C
700143	MT Enzyme Mixture	3 vials/300 µl	-80°C
700144	MT Colorimetric Mixture	1 vial	-20°C
700145	MT Assay AdoHcy Positive Control	1 vial/200 µl	-20°C
700146	MT Assay S-Adenosylmethionine	3 vials	-80°C
700012	HCl Assay Reagent (20 mM)	1 vial/1 ml	-20°C
700020	Half Volume 96-Clear Plate	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

E-Mail: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as specified in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Purified S-adenosyl-L-methionine dependent methyltransferase
2. Appropriate methyltransferase acceptor substrate
3. A plate reader with the ability to measure absorbance at 515 nm
4. Adjustable pipettes and a multichannel or repeating pipette
5. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Methylation of key biological molecules and proteins plays important roles in numerous biological systems, including signal transduction, biosynthesis, protein repair, gene silencing, and chromatin regulation.[1] The S-adenosylmethionine (SAM) dependent methyltransferases use SAM, the second most commonly used enzymatic cofactor after ATP. SAM, also known as AdoMet, acts as a donor of a methyl group that is required for the modification of proteins and DNA.[2] Aberrant levels of SAM have been linked to many abnormalities, including Alzheimer's disease, depression, Parkinson's disease, multiple sclerosis, liver failure, and cancer.[1,2]

About This Assay

Cayman's Methyltransferase Colorimetric Assay Kit is a continuous enzyme-coupled assay that can monitor SAM-dependent methyltransferases.[3] Figure 1, on page 7, outlines the general scheme of the assay. The removal of the methyl group from SAM generates S-adenosylhomocysteine (AdoHcy), which is rapidly converted to S-ribosylhomocysteine and adenine by AdoHcy nucleosidase. This rapid conversion prevents the buildup of AdoHcy and its feedback inhibition on the methylation reaction. Finally, the adenine is converted to hypoxanthine, by adenine deaminase, which in turn is converted to urate and hydrogen peroxide (H_2O_2). The rate of production of H_2O_2 is measured with the colorimetric reagents, 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine, by an increase in absorbance at 515 nm. The assay is supplied with AdoHcy as a positive control. The assay can be used with any purified SAM-dependent methyltransferase.

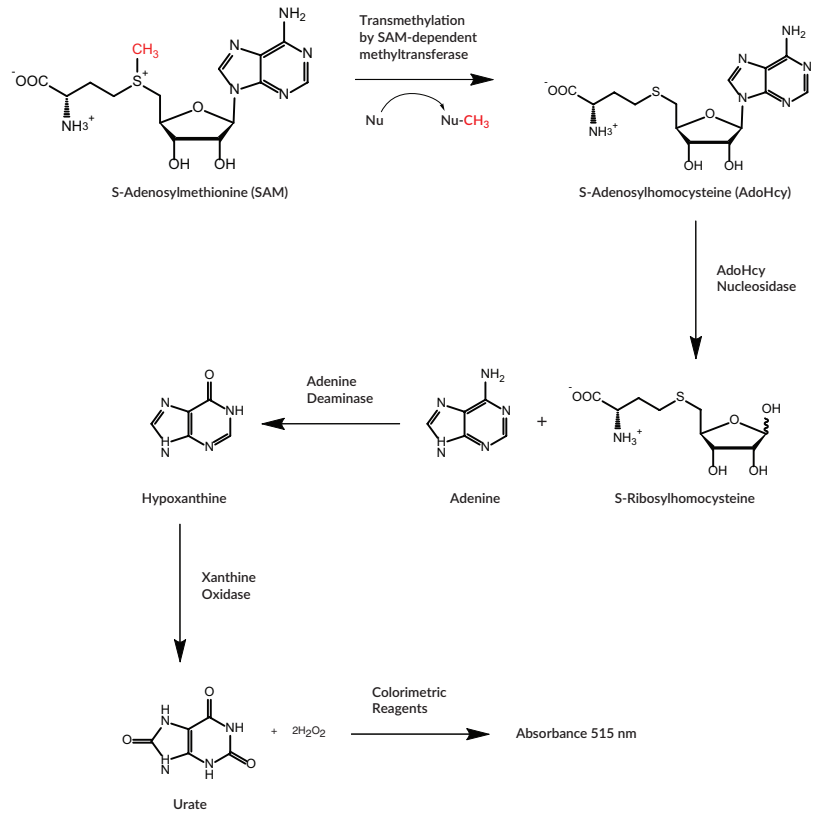


Figure 1. Assay Scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **MT Assay Buffer - (Item No. 700141) and MT Assay Buffer Additive - (Item No. 700142)**

   Thaw the Assay Buffer and Assay Buffer Additive at room temperature. Add the entire volume of the Additive into the Assay Buffer and mix thoroughly. Mark the Additive box on the Assay Buffer vial. Store the Assay Buffer at room temperature; do not freeze after the addition of Additive.

2. **MT Enzyme Mixture - (Item No. 700143)**

   Each vial contains MT enzyme mixture. Thaw only the number of vials you will be using for your experiment on ice. We do not recommend repeated freeze/thaw cycles of the Enzyme Mixture. The Enzyme Mixture is ready to use to prepare the Master Mixture.

3. **MT Colorimetric Mixture - (Item No. 700144)**

   This vial contains a lyophilized powder of 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine. Add 1 ml of Assay Buffer with Additive to the vial and vortex. If not using the entire plate, then the Colorimetric Mixture can be aliquoted into smaller vials and stored at -80°C. The reconstituted Colorimetric Mixture is stable for at least three months at -80°C.

4. **MT Assay AdoHcy Positive Control - (Item No. 700145)**

   This vial contains 200 µl of a 1 mM solution of adenosylhomocysteine (AdoHcy). Thaw the vial on ice. It is ready to use in the assay.

5. **MT Assay S-Adenosylmethionine - (Item No. 700146)**

   Each vial contains lyophilized S-adenosylmethionine (SAM). Reconstitute the contents of the vial with 100 µl of 20 mM HCl (Item No. 700012) to yield 6.9 mM SAM. It is ready to use to prepare the Master Mixture. Prepare additional vials as needed.

6. **HCl Assay Reagent (20 mM) - (Item No. 700012)**

   This vial contains 1 ml of 20 mM hydrochloric acid. The reagent is ready to use as supplied.

Sample Preparation

This assay is suitable for use with all purified SAM-dependent methyltransferases. It is necessary to titrate each enzyme/substrate system in the assay to determine optimal conditions. An example of human lysine specific histone methyltransferase, SET7/9, assayed with 20 µM of the acceptor substrate TAF-10, is shown in Figure 3 on page 15.[4] Avoid the use of reducing agents (including DTT, β-mercaptoethanol, and TCEP) and metal chelators, such as EDTA and EGTA, as these have an inhibitory effect on the reaction. If these reagents are present, dialysis against 0.1 M Tris-HCl, pH 8.0, is recommended.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. We suggest that each sample be assayed at least in duplicate. Two wells should be designated as background wells and two wells should be designated as the positive control. A typical layout of samples to be measured in duplicate is shown in Figure 2 below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	5	5	13	13	21	21	29	29	37	37
B	S	S	6	6	14	14	22	22	30	30	38	38
C	SB	SB	7	7	15	15	23	23	31	31	39	39
D	IA	IA	8	8	16	16	24	24	32	32	40	40
E	1	1	9	9	17	17	25	25	33	33	41	41
F	2	2	10	10	18	18	26	26	34	34	42	42
G	3	3	11	11	19	19	27	27	35	35	43	43
H	4	4	12	12	20	20	28	28	36	36	44	44

PC = AdoHcy Positive Control
S = Sample
SB = Sample Background
IA = Sample + Inhibitor/Activator
1-44 = Other Samples

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 115 µl in all the wells.
- All reagents except the enzymes must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying samples in triplicate, but it is at the user's discretion to do so.
- The assay is performed at 37°C.
- Monitor the absorbance at 515 nm using a plate reader.

Performing the Assay

1. In a suitable tube, prepare the Master Mixture according to the table below:

Reagent	35 wells	70 wells	105 wells
Assay Buffer + Additive	3 ml	6 ml	9 ml
MT Enzyme Mixture	250 µl	500 µl	750 µl
MT Colorimetric Mixture	200 µl	400 µl	600 µl
MT SAM	1 vial/100 µl	2 vials/200 µl	3 vials/300 µl

Table 1. Master Mixture Preparation

2. **AdoHcy Positive Control Wells -** add 10 µl of MT Assay Buffer and 5 µl of Positive Control in the designated wells on the plate (see **Sample plate format**, Figure 2, on page 10).
3. **Sample Wells -** add 5 µl of sample to at least two wells. To obtain reproducible results, the amount of methyltransferase added to the wells should fall within the range of the assay. When necessary, samples should be diluted with Assay Buffer or 0.1 M Tris-HCl, pH 8.0, to bring the enzymatic activity to this level.
4. **Sample Background Wells -** add 5 µl of MT Assay Buffer to two wells.
5. Add 10 µl of the appropriate acceptor substrate to only the sample and sample background wells.
6. Initiate the reactions by quickly adding 100 µl of Master Mixture to the Positive Control, sample, and sample background wells.
7. Immediately read the absorbance at 515 nm every 30 seconds to one minute at 37°C until the increasing absorbances plateau (approximately 15-30 minutes).

Well	Assay Buffer	Positive Control	Sample	Acceptor	Master Mix
Positive Control	10 µl	5 µl	-	-	100 µl
Sample	-	-	5 µl	10 µl	100 µl
Background	5 µl	-	-	10 µl	100 µl

Table 2. Pipetting summary

NOTE: If assaying inhibitors or activators, adjust the enzyme and acceptor substrate concentration so that all three components are added to the assay in a final volume of 15 µl (i.e., 5 µl methyltransferase, 5 µl inhibitor/activator, and 5 µl acceptor substrate). Keep the methyltransferase volume at 5 µl.

ANALYSIS

Calculations

1. Determine the average absorbance of each sample.
2. Determine the change in absorbance (ΔA) per minute:
   a) Plot the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve (an example of human lysine specific histone methyltransferase, SET7/9, assayed with 20 µM of the acceptor substrate TAF-10, is shown in Figure 3 on page 15)

   OR

   b) Select two points on the linear portion of the curve and determine the change in absorbance during that time using the following equation:

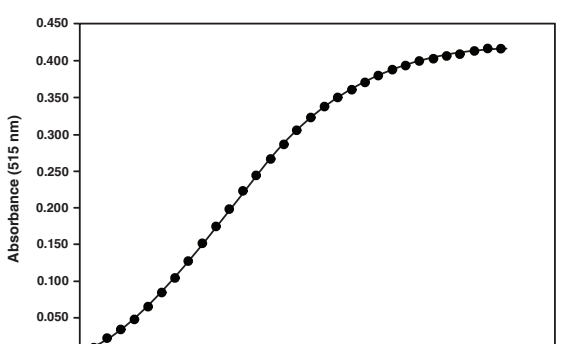
$$\Delta A/min = \frac{A\ (Time\ 2) - A\ (Time\ 1)}{Time\ 2\ (min) - Time\ 1\ (min)}$$

3. Determine the rate of ΔA/min for the sample background wells and subtract this rate from that of the sample wells.
4. Use the following formula to calculate the methyltransferase activity. The reaction rate can be determined using the extinction coefficient of the product of the 3,5-dichloro-2-hydroxybenzenesulfonic acid reaction with hydrogen peroxide and 4-aminoantipyrine*. One unit of methyltransferase will transfer 1.0 µmol of a methyl group per minute at 37°C.

Methyltransferase Activity (µmol/min/ml) =

$$\frac{\Delta A/min}{16.9\ mM^{-1}} \times \frac{0.115\ ml}{0.005\ ml} \times \text{Sample dilution}$$

*The actual extinction coefficient is 26.0 $mM^{-1}cm^{-1}$. This value has been adjusted for the pathlength of the solution in the well (0.65 cm).



Human lysine specific histone methyltransferase SET7/9 assayed with 20 µM TAF-10 as the acceptor substrate

Figure 3. Human lysine specific histone methyltransferase SET7/9 assayed with 20 µM TAF-10 as the acceptor substrate.

5. If inhibitors or activators were assayed, determine the percent inhibition/activation for each sample by subtracting the activity of each inhibitor/activator sample from the activity of its corresponding non-treated sample. Divide the result by the activity of the non-treated sample, and multiply by 100 to give the percent inhibition/activation.

Performance Characteristics

Assay Range:

The detection range of this assay is from 0.013-0.133 µmol/min/ml of methyltransferase activity which is equivalent to an absorbance increase of 0.01 to 0.1 per minute.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No absorbance detected above background in the sample wells	Sample was too dilute or acceptor substrate was not added	Re-assay using a more concentrated sample, and make sure the appropriate acceptor substrate is added
The color development was too fast	Too much enzyme was added to the wells	Dilute your samples with assay buffer or 0.1 M Tris-HCl, pH 8.0, and re-assay
No inhibition/activation was seen with compound	A. The compound concentration is not high enough B. The compound is not an inhibitor/activator of the enzyme	Increase the compound concentration and re-assay

References

1. Loenen WAM. S-Adenosylmethionine: Jack of all trades and master of everything? *Biochem Soc Trans* **34(2)**, 330-333 (2006).
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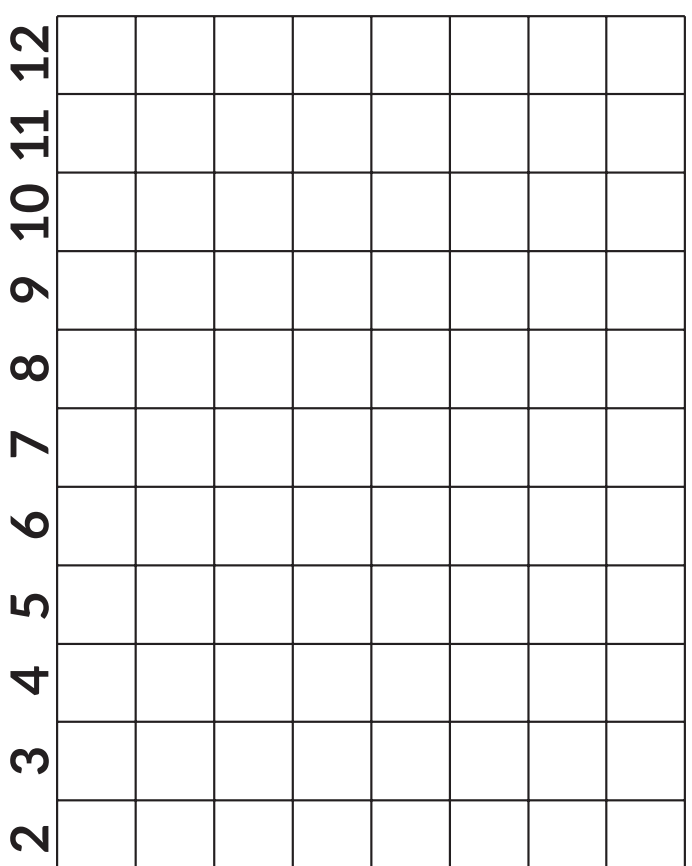
4. Couture, J.-F., Collazo, E., Hauk, G., *et al.* Structural basis for the methylation site specificity of SET7/9. *Nature Structural and Molecular Biology* **13(2)**, 140-146 (2006).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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