

**SET7/9 Methyltransferase Inhibitor  
Screening Assay Kit**

Item No. 700270

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## GENERAL INFORMATION

### Materials Supplied

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

Item Number	Item	Quantity/Size	Storage
700141	MT Assay Buffer	1 vial/20 ml	-80°C
700142	MT Assay Buffer Additive	1 vial/0.2 ml	-80°C
700143	MT Enzyme Mixture	3 vials/0.3 ml	-80°C
700151	MT Fluorometric Mixture	3 vials/lyophilized	-80°C
700146	MT Assay SAM	3 vials/0.2 ml	-80°C
700271	SET7/9 (human recombinant)	2 vials/0.1 ml	-80°C
700153	MT DMSO	1 vial/1 ml	-80°C
700272	SET7/9 Acceptor Peptide	2 vials/0.6 ml	-20°C
700145	MT Assay AdoHcy Positive Control	1 vial/200 µl	-80°C
400091	Half Volume 96-Well Solid Plate (black)	1 plate	Room temperature
400012	96-Well Cover Sheet	1 cover	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



**WARNING:** This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

## Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

## If You Have Problems

### Technical Service Contact Information

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

**Fax:** 734-971-3641

**Email:** techserv@caymanchem.com

**Hours:** M-F 8:00 AM to 5:30 PM EST

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 at -80°C and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader with the capacity to measure fluorescence using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm
2. Adjustable pipettes and a repeat pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

## INTRODUCTION

### Background

Methylation of key biological molecules plays important roles in numerous systems, including signal transduction, biosynthesis, protein repair, gene silencing, and chromatin regulation.<sup>1</sup> The S-adenosylmethionine (SAM)-dependent methyltransferases use SAM, also known as AdoMet, as a methyl group donor for the modification of both proteins and DNA.<sup>2</sup> Aberrant levels of SAM have been linked to many abnormalities, including Alzheimer's Disease, depression, Parkinson's Disease, multiple sclerosis, liver failure, and cancer.<sup>1,2</sup>

SET Domain-containing Protein 7/9 (SET7/9; lysine methyltransferase 7 (KMT7)) is a methyltransferase that acts on various substrates including histone 3 at lysine residue 4 (H3K4), p53, and the transcription factor TAF 10.<sup>3</sup> Lysine residues can be mono-, di-, or tri-methylated. Unlike most SET proteins, SET7/9 is exclusively a mono-methylase.<sup>4</sup> Methylation of lysine residues can promote transcriptional activation or repression and is critical for regulating histone function.<sup>5</sup> SET7/9 methylation of p53 in response to DNA damage activates p53 for subsequent acetylation.<sup>5</sup> SET7/9 is able to modulate p53 activity in a human cancer cell line, implying that it may play a significant role in human tumorigenesis.

### About This Assay

Cayman's SET7/9 Methyltransferase Inhibitor Screening Assay provides a convenient method for screening human SET7/9 inhibitors. Figure 1 outlines the general scheme of the assay.<sup>6</sup> The transfer of the methyl group from SAM by SET7/9 to the acceptor peptide (TAF 10) generates S-adenosylhomocysteine, which is rapidly converted to S-ribosylhomocysteine and adenine by adenosylhomocysteine nucleosidase. This rapid conversion prevents the buildup of adenosylhomocysteine 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<sub>2</sub>O<sub>2</sub>). The reaction between H<sub>2</sub>O<sub>2</sub> and ADHP (10-acetyl-3,7-dihydroxyphenoxazine) produces the highly fluorescent compound resorufin. Resorufin fluorescence is analyzed using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

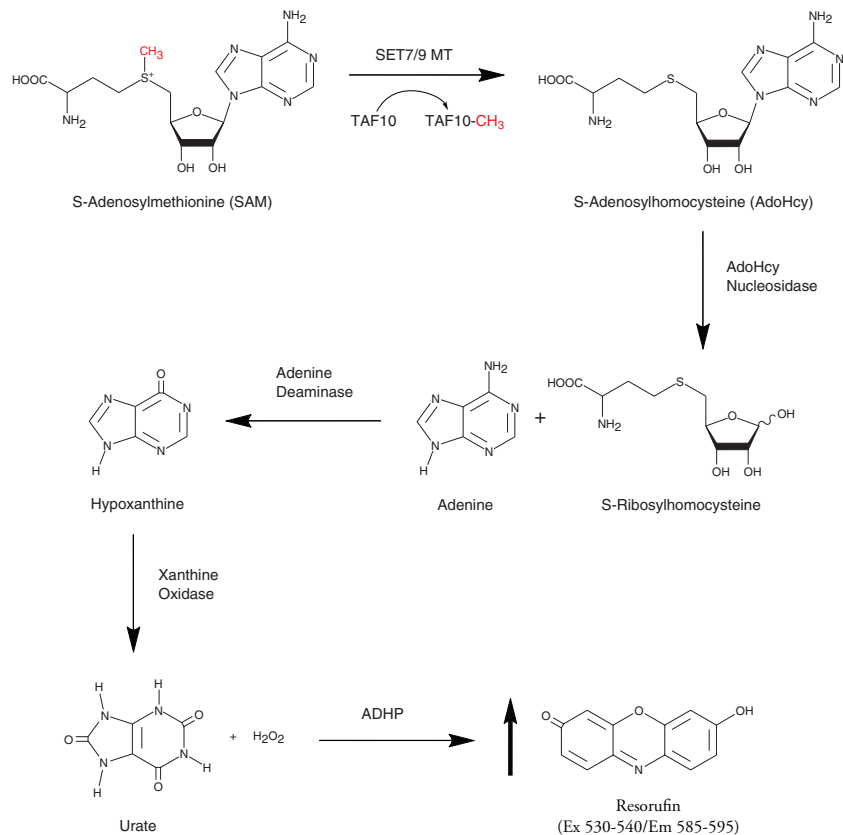


Figure 1. Assay scheme

## PRE-ASSAY PREPARATION

### Reagent Preparation

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

Thaw the MT Assay Buffer and MT 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 300  $\mu$ l of enzyme mixture. Thaw on ice only the number of vials you will be using for your experiment. 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 Fluorometric Mixture - (Item No. 700151)

The vials contain a clear lyophilized powder of 10-acetyl-3,7-dihydroxyphenoxazine (ADHP). Immediately prior to making the Master mixture, add 100  $\mu$ l of MT DMSO (Item No. 700153) to the vial and vortex. Then add 400  $\mu$ l of Assay Buffer containing Additive and vortex. Prepare additional vials as needed. The reconstituted Mixture is stable for 45 minutes. After 45 minutes, increased background fluorescence will occur.

#### 4. MT Assay SAM - (Item No. 700146)

Each vial contains 200  $\mu$ l of S-adenosylmethionine (SAM). Thaw on ice only the number of vials you will be using for your experiment. We do not recommend repeated freeze/thaw cycles. SAM is ready to use to prepare the Master Mixture.

**5. SET7/9 (human recombinant) - (Item No. 700271)**

Each vial contains 100  $\mu\text{l}$  of human recombinant SET7/9 methyltransferase (N-terminal His-tagged SET7/9 amino acids 1-366). Thaw the enzyme on ice. Prior to assaying, add 500  $\mu\text{l}$  of Assay Buffer containing additive to the vial. This is enough enzyme for assaying 60 wells. Dilute the additional vial if assaying the entire plate. The diluted enzyme is stable for four hours on ice.

**6. MT DMSO - (Item No. 700153)**

The vial contains 1 ml of dimethylsulfoxide (DMSO). The reagent is ready to use as supplied.

**7. SET7/9 Acceptor Peptide - (Item No. 700272)**

Each vial contains 0.6 ml of 438  $\mu\text{M}$  human TAF 10 peptide (Ac-SKSKDRKYTL). The peptide is ready to use in the assay. *NOTE: The final concentration of peptide in the assay as described is 35  $\mu\text{M}$ . This concentration may be reduced with Assay Buffer at the user's discretion. The  $K_m$  value for the peptide is 43  $\mu\text{M}$ .*

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

The vial contains 200  $\mu\text{l}$  of a 1 mM solution of adenosylhomocysteine (AdoHcy). The AdoHcy can be used to assay for interference (see page 15).

**Plate Set Up**

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% Initial Activity wells and three wells designated as background wells. We suggest that each inhibitor be assayed in triplicate and that you record the contents of each well on the template sheet provided on page 19. A typical layout of samples and inhibitors to be measured in triplicate is shown in Figure 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells

A - 100% Initial Activity Wells

1-30 - Inhibitor Wells

**Figure 2. Sample plate format**

### Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps 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 125  $\mu\text{l}$  in all the wells.
- All reagents except the enzyme 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 the user's discretion to do so.
- The assay is performed at 37°C.
- Monitor the fluorescence with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

## Performing the Assay

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

Reagent	36 wells	72 wells	100 wells
MT Assay Buffer + Additive	2.9 ml	5.8 ml	8.7 ml
MT Enzyme Mixture	1 vial or 300 $\mu\text{l}$	2 vials or 600 $\mu\text{l}$	3 vials or 900 $\mu\text{l}$
MT Fluorometric Mixture	200 $\mu\text{l}$	400 $\mu\text{l}$	600 $\mu\text{l}$
MT SAM	1 vial or 200 $\mu\text{l}$	2 vials or 400 $\mu\text{l}$	3 vials or 600 $\mu\text{l}$

**Table 1. Master Mixture Preparation**

2. **100% Initial Activity Wells** - add 100  $\mu\text{l}$  of Master Mixture, 10  $\mu\text{l}$  of SET7/9 Acceptor Peptide, and 5  $\mu\text{l}$  of solvent (same solvent used to dissolve the inhibitor) to three wells.
3. **Background Wells** - add 100  $\mu\text{l}$  of Master Mixture, 10  $\mu\text{l}$  of SET7/9 Acceptor Peptide, and 5  $\mu\text{l}$  of solvent (same solvent used to dissolve the inhibitor) to three wells.
4. **Inhibitor Wells** - add 100  $\mu\text{l}$  of Master Mixture, 10  $\mu\text{l}$  of SET7/9 Acceptor Peptide, and 5  $\mu\text{l}$  of inhibitor\* to three wells.

	Master Mixture	MT Acceptor Peptide	Solvent	Inhibitor
100% Initial Activity	100 µl	10 µl	5 µl	
Background	100 µl	10 µl	5 µl	
Inhibitor	100 µl	10 µl		5 µl

**Table 2. Pipetting summary**

- Initiate the reactions by adding 10 µl of SET7/9 to the 100% Initial Activity and Inhibitor wells and add 10 µl of Assay Buffer to the background wells.
- Cover the plate with the plate cover and incubate for twenty minutes at 37°C.
- Remove the plate cover and read at an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

\*Inhibitors can be dissolved in Assay Buffer, methanol, DMSO, or ethanol and should be added to the assay in a final volume of 5 µl. In the event that an appropriate concentration of inhibitor is completely unknown, we recommend that several dilutions of the inhibitor be made. For determination of IC<sub>50</sub> values, use additional concentrations of inhibitor to cover a larger range.

## ANALYSIS

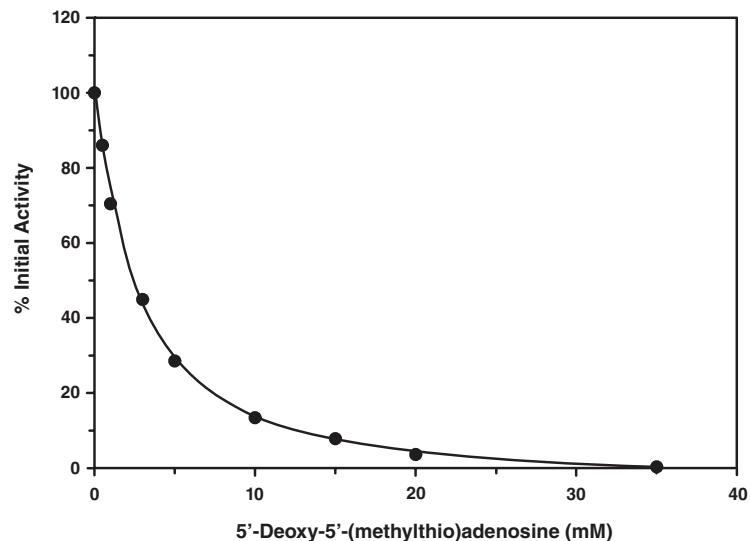
### Calculations

- Determine the average fluorescence of the background, 100% initial activity (IA), and inhibitor wells.
- Subtract the average fluorescence of the background wells from the average fluorescence of the 100% initial activity and inhibitor wells.
- Determine the percent inhibition or percent Initial Activity for each inhibitor using one of the following equations.

$$\% \text{ Inhibition} = \left[ \frac{\text{IA} - \text{Inhibitor}}{\text{IA}} \right] \times 100$$

$$\% \text{ Initial Activity} = \frac{\text{Inhibitor}}{\text{IA}} \times 100$$

- Graph the percent inhibition or percent initial activity as a function of the inhibitor concentration to determine the IC<sub>50</sub> value (concentration at which there was 50% inhibition). The inhibition of human recombinant SET7/9 MT by the broad spectrum methyltransferase inhibitor, 5'-deoxy-5'-(methylthio)adenosine, is shown in Figure 3 (see page 14) as an example.<sup>7</sup>



**Figure 3. Inhibition of human recombinant SET7/9 MT by 5'-deoxy-5'-(methylthio)adenosine ( $IC_{50} \sim 2.5$  mM)**

## Performance Characteristics

### Precision:

When a series of 16 SET7/9 measurements were assayed on the same day, the intra-assay coefficient of variation was 1.3%. When a series of 16 SET7/9 measurements were assayed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 1.5%.

## RESOURCES

### Interferences

It is possible that a compound tested for SET7/9 inhibition will interfere with the downstream enzymes in the assay. Potential interference can be tested by assaying the compound in question with the AdoHcy Positive Control. A procedure is outlined below.

### Testing for Interference

1. Thaw the AdoHcy Positive Control (Item No. 700145) on ice. Dilute 10  $\mu$ l of AdoHcy with 190  $\mu$ l of Assay Buffer containing Additive.
2. **AdoHcy wells** - add 100  $\mu$ l of Master Mixture and 5  $\mu$ l of solvent (the same solvent used to dissolve the compound) to three wells.
3. **Compound wells** - add 100  $\mu$ l of Master Mixture, 5  $\mu$ l of compound to three wells.
4. Initiate the reactions by adding 10  $\mu$ l of diluted AdoHcy to the AdoHcy wells and the compound wells.
5. Cover the plate with the plate cover and incubate for 10 minutes at 37°C.
6. Remove the plate cover and read the plate at an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. It may be necessary to adjust the gain setting on the instrument to allow for the measurement of all the samples.

### Calculating the Percent Interference

1. Determine the average fluorescence of the AdoHcy and the compound wells.
2. Determine the percent interference for the compound. To do this, subtract each compound value from the AdoHcy value. Divide the result by the AdoHcy value and then multiply by 100 to give the percent interference. The percent interference should be less than 10% for the compound to be not affecting the assay.

## 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 A. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence was detected above background in the inhibitor wells	A. Enzyme or acceptor was not added to the well(s) B. Inhibitor concentration is too high and inhibited all of the enzyme activity	A. Make sure to add all of the components to the wells B. Reduce the concentration of the inhibitor and re-assay
Fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read
No inhibition was seen with inhibitor	A. The inhibitor concentration is not high enough B. The inhibitor is not an inhibitor of the enzyme	Increase the inhibitor concentration and re-assay

## References

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- Song, M.-R., Ghosh, A., FGF2-induced chromatin remodeling regulates CNTF-mediated gene expression and astrocyte differentiation. *Nature Neuroscience* **7(3)**, 229-235 (2004)

## Related Products

HAT Inhibitor Screening Assay Kit - Item No. 10006515  
 HDAC Activity Assay Kit - Item No. 10011563  
 HDAC1 Inhibitor Screening Assay Kit - Item No. 10011564  
 LSD1 Inhibitor Screening Assay Kit - Item No. 700120  
 Methyltransferase Colorimetric Assay Kit - Item No. 700140  
 Methyltransferase Fluorometric Assay Kit - Item No. 700150  
 SET7/9 (human recombinant) - Item No. 10320  
 SET7/9 Polyclonal Antibody - Item No. 13731  
 SIRT1 FRET-Based Screening Assay Kit - Item No. 10010991  
 SIRT1 Direct Fluorescent Screening Assay Kit - Item No. 10010401  
 SIRT2 Direct Fluorescent Screening Assay Kit - Item No. 700280  
 SIRT3 Direct Fluorescent Screening Assay Kit - Item No. 10011566  
 SIRT6 Direct Fluorescent Screening Assay Kit - Item No. 700290

## Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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## NOTES

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