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- Mindermengenzuschlag
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# SET7/9 Methyltransferase Inhibitor Screening Assay Kit

Item No. 700270

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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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 | 96 wells Quantity/Size | 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 |
| 700002 | ADHP Assay Reagent | 3 vials | -20°C |
| 700146 | MT Assay S-Adenosylmethionine | 3 vials | -80°C |
| 700271 | SET7/9 (human recombinant) | 2 vials/100 µl | -80°C |
| 700001 | DMSO Assay Reagent | 1 vial/1 ml | RT |
| 700012 | HCl Assay Reagent (20 mM) | 1 vial/1 ml | -20°C |
| 700272 | SET7/9 Acceptor Peptide | 2 vials/600 µl | -20°C |
| 700145 | MT Assay AdoHcy Positive Control | 1 vial/200 µl | -80°C |
| 400091 | Half-Volume 96-Well Solid Plate (black) | 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.**

The reagents in this kit have been tested and formulated to work exclusively with Cayman's SET7/9 SAM-Screener™ Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

**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

**Email:** 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 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. 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 repeating 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.[1] 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.[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]

SET Domain-containing Protein 7/9 (SET7/9; lysine methyltransferase 7 (KMT7) and SETD7/9) is a methyltransferase that acts on various substrates including histone 3 at lysine residue 4 (H3K4), p53, and the transcription factor TAF 10.[3] Lysine residues can be mono-, di-, or tri-methylated. Unlike most SET proteins, SET7/9 is exclusively a mono-methylase.[4] Methylation of lysine residues can promote transcriptional activation or repression and is critical for regulating histone function.[5] SET7/9 methylation of p53 in response to DNA damage activates p53 for subsequent acetylation.[5] SET7/9 is able to modulate p53 activity in a human cancer cell line, implying that it may play a significant role in human turmorigenesis.



## 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.[6] 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 ($H_2O_2$). The reaction between $H_2O_2$ 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.

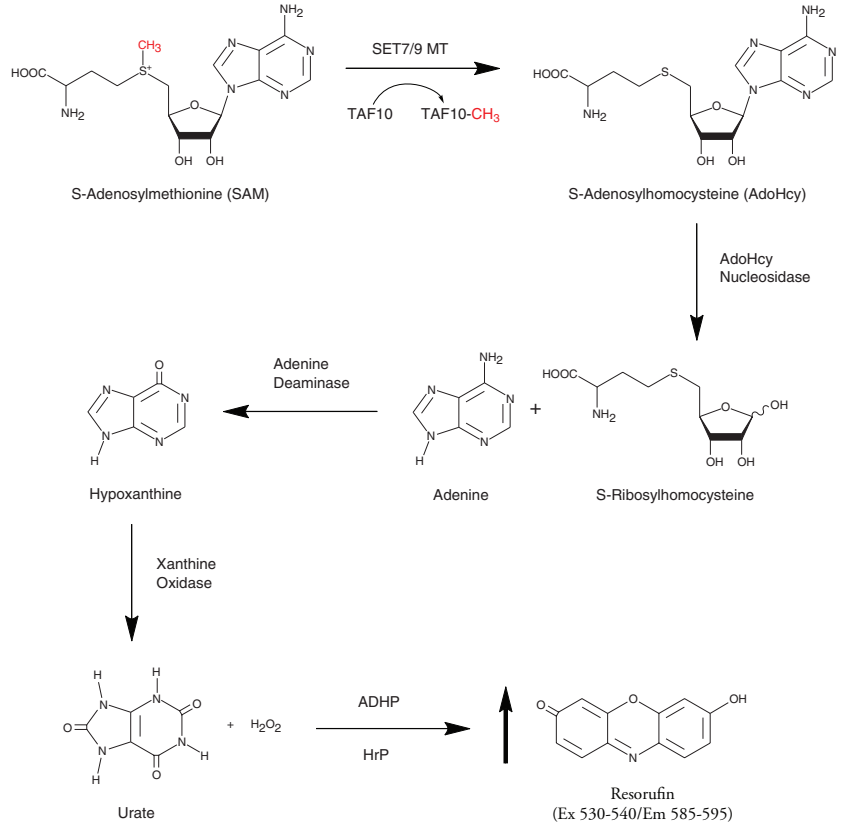



**Figure 1. Assay scheme**



# PRE-ASSAY PREPARATION

*NOTE: Water used to prepare all reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for FP. UltraPure water may be purchased from Cayman Chemical (Item No. 400000).*

## Buffer 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 µl 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. **ADHP Assay Reagent - (Item No. 700002)**

   The vials contain a lyophilized powder of 10-acetyl-3,7-dihydroxyphenoxazine (ADHP). Immediately prior to making the Master mixture, add 100 µl of DMSO Assay Reagent (Item No. 700001) to the vial and vortex. Then add 400 µl of **HPLC-grade water** and vortex. Prepare additional vials as needed. The reconstituted Mixture is stable for 60 minutes. After 60 minutes, increased background fluorescence will occur.

4. **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.

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

   Each vial contains 100 µl 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 µl 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. **DMSO Assay Reagent - (Item No. 700001)**

   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 µM 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 µM. This concentration may be reduced with Assay Buffer at the user's discretion. The $K_m$ value for the peptide is 43 µM.*

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

   The vial contains 200 µl of a 1 mM solution of adenosylhomocysteine (AdoHcy). The AdoHcy can be used to assay for interference (see page 15).

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

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



# ASSAY PROTOCOL

## 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 18. 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**

## Performing the Assay

**Pipetting Hints**

- Use different tips to pipette each reagent.
- Do not expose the pipette tip to the reagent(s) already in the well.
- Avoid introducing bubbles into the wells.

### General Information

- The final volume of the assay is 125 µl 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.

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

| Reagent | 36 wells | 72 wells | 100 wells |
|---|---|---|---|
| Assay Buffer + Additive | 3 ml | 6 ml | 9 ml |
| MT Enzyme Mixture | 1 vial/300 µl | 2 vials/600 µl | 3 vials/900 µl |
| ADHP Assay Reagent | 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. **100% Initial Activity Wells** - add 100 µl of Master Mixture, 10 µl of SET7/9 Acceptor Peptide, and 5 µl of solvent (same solvent used to dissolve the inhibitor) to three wells.
3. **Background Wells** - add 100 µl of Master Mixture, 10 µl of SET7/9 Acceptor Peptide, and 5 µl of solvent (same solvent used to dissolve the inhibitor) to three wells.
4. **Inhibitor Wells** - add 100 µl of Master Mixture, 10 µl of SET7/9 Acceptor Peptide, and 5 µl 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**

5. 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.
6. Cover the plate with the plate cover and incubate for twenty minutes at 37°C.
7. 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 $IC_{50}$ values, use additional concentrations of inhibitor to cover a larger range.

# ANALYSIS

## Calculations

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

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

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

4. Graph the percent inhibition or percent initial activity as a function of the inhibitor concentration to determine the $IC_{50}$ value (concentration at which there was 50% inhibition).

## 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 µl of AdoHcy with 190 µl of Assay Buffer containing Additive.
2. **AdoHcy wells -** add 100 µl of Master Mixture and 5 µl of solvent (the same solvent used to dissolve the compound) to three wells.
3. **Compound wells -** add 100 µl of Master Mixture, 5 µl of compound to three wells.
4. Initiate the reactions by adding 10 µl 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.

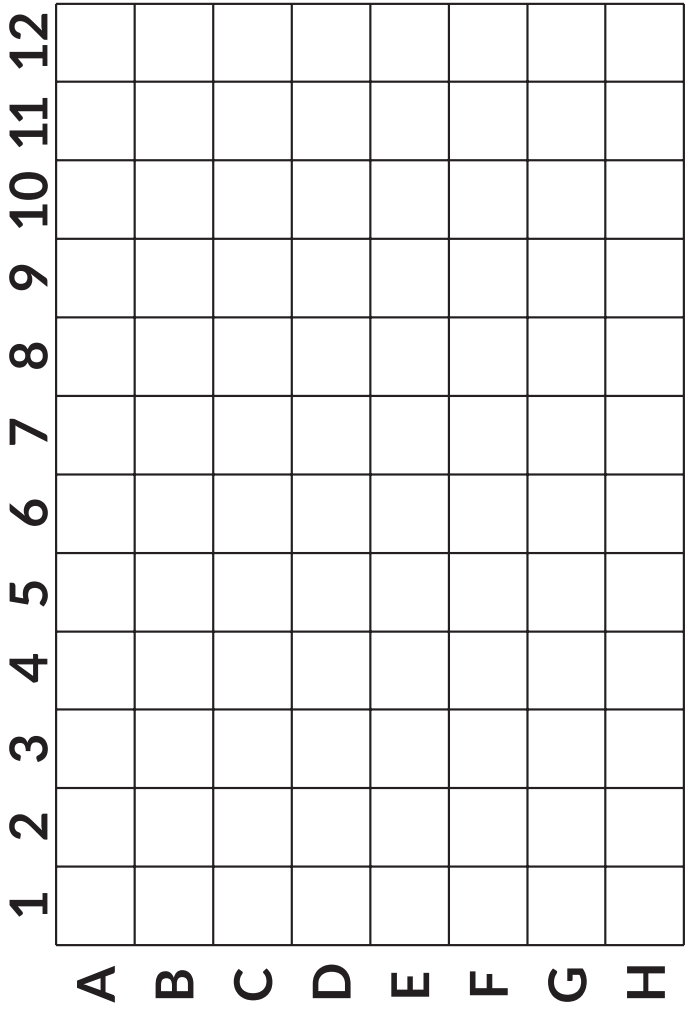
## Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|---|
| Erratic values; dispersion of duplicates | A. Bubble in the well(s)<br>B. Poor pipetting/technique | A. Be careful not to splash the contents of the wells<br>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)<br>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<br>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<br>B. The inhibitor is not an inhibitor of the enzyme | Increase the inhibitor concentration and re-assay |



## References


1. Loenen, W.A.M. S-Adenosylmethionine: Jack of all trades and master of everything? *Biochem. Soc. Trans.* **34(2)**, 330-333 (2006).
2. Chiang, P.K., Gordon, R.K., Tal, J., *et al.* S-adenosylmethionine and methylation. *FASEB J.* **10**, 471-480 (1996).
3. 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).
4. Xiao, B., Jing, C., Wilson, J.R., *et al.* Structure and catalytic mechanism of the human histone methyltransferase SET7/9. *Nature* **421**, 652-656 (2003).
5. Kurash, J.K., Lei, H., Shen, Q., *et al.* Methylation of p53 by Set7/9 mediates p53 acetylation and activity *in vivo*. *Molecular Cell* **29**, 392-400 (2008).
6. Dorgan, K.M., Wooderchak, W.L., Wynn, D.P., *et al.* An enzyme-coupled continuous spectrophotometric assay for S-adenosylmethionine-dependent methyltransferases. *Anal. Biochem.* **350**, 249-255 (2006).






## NOTES

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