



Reagents require separate storage conditions.



Check our website for additional protocols, technical notes and FAQs.



For proper performance, use the insert provided with each individual kit received.

## Methyltransferase Activity Kit

Catalog #: ADI-907-032

Sufficient Reagents for 96 tests  
For use with purified *in vitro* samples

## **USE FOR RESEARCH PURPOSES ONLY**

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

## **LIMITED WARRANTY; DISCLAIMER OF WARRANTIES**

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

## **TRADEMARKS AND PATENTS**

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**



Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.



Please contact Enzo Life Sciences Technical Support if necessary.

## TABLE OF CONTENTS

Introduction .....	2
Principle .....	2
Materials Supplied .....	3
Storage .....	4
Other Materials Needed .....	4
Sample Handling .....	5
Reagent Preparation .....	6
Assay Procedure .....	8
Example of End Point Assay Results .....	9
Example SAH Titration Results .....	10
Typical Inhibition Assay Procedure .....	11
Calculation of Results .....	12
Typical Inhibition Assay Results .....	13
Typical Enzyme Interference Assay Procedure .....	14
Interfering Substances .....	15
References .....	16
Contact Information .....	18

## INTRODUCTION

The Methyltransferase HT activity kit is a complete kit for the high throughput screening of candidate compounds that may alter the activity of S-adenosylmethionine (SAM)-dependent methyltransferases. In this kit SAM is provided separately from the Reaction Buffer, allowing the investigator to customize the concentration needed for each methyltransferase of interest. Please read the complete kit insert before performing this assay.

Methylation of proteins, nucleic acids and oligosaccharides is an important post-translational regulatory event. Activities that are methylation-related include meiosis, biosynthesis, development, signal transduction, chromatin regulation, and gene silencing.<sup>1-9</sup> The enzymes that mediate the covalent transfer of a methyl group from a donor to an acceptor molecule are methyltransferases. Methyltransferases have structurally unrelated acceptors as diverse as proteins and DNA, however frequently use S-adenosylmethionine as a universal donor.<sup>10</sup> Part of the acceptor diversity of this enzyme family relates to the flexible structural folds that bind these molecules in proximity of the donor.<sup>11</sup> The side-chains of lysine, arginine, glutamate, glutamine, asparagines, and isoprenylated residues serve as methylation sites in proteins like histones.<sup>1</sup> Changes in methylation patterns have been tightly linked to disease states such as cancer and vascular disease.<sup>12-14</sup>

## PRINCIPLE

1. Methyltransferase, detection solution, and reaction mix spiked with SAM and acceptor substrate are added to wells of a black 96-well plate. The plate is then incubated to allow the reaction to proceed.
2. The reaction is quenched with an appropriate stop solution.
3. The plate is transferred to a plate reader and fluorescence is measured at 380ex/520em.

**Methyltransferase**

**Acceptor Substrate+Reaction Buffer+Detection Solution→Fluorescence**



Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.



Detection Solution contains DMSO. Avoid skin contact. Avoid breathing vapor.



The physical, chemical, and toxicological properties of the chemicals and reagents contained in this kit may not have been fully investigated. Therefore, we recommend the use of gloves, lab coats, and eye protection while using any of these chemical reagents.

## MATERIALS SUPPLIED

- 1. Black Microtiter Plate**  
One plate of 96 wells, Catalog No. 80-1675  
The plate is ready to use.
- 2. Transferase Assay Buffer Concentrate**  
15ml, Catalog No. 80-1648  
A 10X concentrated buffer containing detergent and preservative.
- 3. SAM-Free Methyltransferase Reaction Buffer Concentrate**  
0.3ml, Catalog No. 80-2067  
A 10X concentrated buffer containing secondary enzymes.
- 4. Transferase Detection Solution Concentrate.**  
0.15ml, Catalog No. 80-1650  
A 100X concentrated solution of fluorescent substrate in DMSO.
- 5. Methyltransferase Positive Control**  
0.2ml, Catalog No. 80-1652  
A buffered solution of 1mM S-(5'-Adenosyl)-L-homocysteine<sub>[TP1]</sub>.
- 6. S-adenosylmethionine**  
0.5ml, Catalog No. 80-2068  
A buffered solution of 1mM S-adenosylmethionine.
- 7. Foil Plate Sealer**  
3 each, Catalog No. 10-3126
- 8. Methyltransferase Assay Layout Sheet**  
1 each, Catalog No. 30-0279



Reagents require separate storage conditions.



If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

## STORAGE

All reagents should be stored at -20°C, with the exception of Transferase Assay Buffer Concentrate which should be stored at 4°C, until the kit expiration date. The recommended storage temperature does not necessarily reflect shipping conditions.

## OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. S-adenosylmethionine dependent methyltransferase.
3. Appropriate methyltransferase acceptor substrate.
4. Inhibitor/activator compounds to be screened.
5. Precision pipets for volumes between 5µl and 1,000µl.
6. Disposable beakers for diluting buffer concentrates.
7. Graduated cylinders.
8. A microplate shaker.
9. Disposable microtubes, 0.5 and 1.5ml.
10. Microplate reader capable of measuring fluorescence at 380ex/520em.
11. Crushed ice and container.
12. An appropriate stop solution, such as isopropyl alcohol (ice cold), 1mM NEM, or 5mM Adenine 9β Arabinoside.

## SAMPLE HANDLING



Before screening colored compounds, the compound should be titrated in the assay to ensure that there is no interference with signal collection.

This assay is suitable for use with all S-adenosyl-L-methionine dependent methyltransferases. It is necessary to titrate SAM and each enzyme/substrate system in the assay to determine optimal conditions.

This assay should only be used to screen purified in vitro samples in buffer systems without reductants.

We recommend that an end-point assay be performed to determine the optimal concentration of SAM needed for the assay. Make serial dilutions of SAM in the Reaction Mix containing the acceptor substrate. Initial concentrations of 80 $\mu$ M are recommended. A kinetic assay format is also an available option.

The positive control provided may also be used to assess the tolerance of the assay for compounds that may interfere with data collection. Please see the Interfering Substances section for known interferants.



Thaw Detection Solution Concentrate at room temperature. Do not place on ice. Thaw all other reagents on ice.



Pre-rinse each pipet tip with reagent.



Store 1X Detection Solution on ice and use within 4 hours of preparation. Discard unused 1X Solution.



Detection Solution contains DMSO. Avoid skin contact. Avoid breathing vapor.

## REAGENT PREPARATION

### 1. Transferase Assay Buffer

Prepare the assay buffer by diluting 15ml of the supplied Transferase Assay Buffer Concentrate with 135ml of deionized water. This solution can be stored at 4°C for 3 months, or the kit's expiration, whichever is earlier. The 1X assay buffer is used to prepare dilutions of Methyltransferase Reaction Mix, Transferase Detection Solution, enzymes, substrates, and compounds to be screened.

### 2. Transferase Detection Solution

Count the total number of wells needed for compound screening and add 6 (for the zero, positive control, and blank wells in duplicate). Use the following formula to calculate the volume of 1X Detection Solution required.

#### A. Total volume required

$$[\text{Total number of wells needed} + 6] \times 100\mu\text{l} = \text{_____}\mu\text{l}$$

#### B. Volume of Transferase Detection Solution Concentrate required

$$[\text{Total volume required (from A. above)}] \times 0.01 = \text{_____}\mu\text{l}$$

#### C. Volume of 1X Transferase Assay Buffer required

$$[\text{Total volume required (from A. above)}] \times 0.99 = \text{_____}\mu\text{l}$$

Prepare 1X Detection Solution by combining the appropriate reagent volumes calculated in B and C above. For example, to prepare 2ml of 1X Detection Solution, combine the following volumes: 20 $\mu\text{l}$  of the supplied Transferase Detection Solution Concentrate and 1980 $\mu\text{l}$  1X Transferase Assay Buffer. Diluted Detection Solution should be kept on ice and used within 4 hours of preparation. Any unused 1X Detection Solution should be discarded.

### 3. Methyltransferase Reaction Mix

Count the total number of wells needed for compound screening and add 6 (for the zero, positive control, and blank wells in duplicate). Use the following formula to calculate the volume of 1X Methyltransferase Reaction Mix required.

#### A. Total volume required

$$[\text{Total number of wells needed} + 6] \times 25\mu\text{l} = \text{_____}\mu\text{l}$$





Store 1X Reaction mix on ice and use within 4 hours of preparation. Discard unused 1X solution.



Thaw Methyltransferase Positive Control on ice.



Store diluted Positive Control on ice and use within 8 hours of preparation. Discard unused 1X solution.

B. Volume of SAM-Free Methyltransferase Reaction Buffer Concentrate required

$$[\text{Total volume required (from A. above)}] \times 0.1 = \text{_____} \mu\text{l}$$

C. Volume of acceptor substrate required

$$[\text{Determined empirically based on enzyme used}] = \text{_____} \mu\text{l}$$

D. Volume of 1X Transferase Assay Buffer required

$$[\text{Total volume required (from A. above)}] \times 0.90 - [\text{volume of substrate required}] = \text{_____} \mu\text{l}$$

Prepare 1X Reaction Mix on ice by combining the appropriate reagent volumes calculated in B, C and D above. For example, to prepare 2ml of Reaction Mix spiked with 22 $\mu\text{l}$  of acceptor substrate, combine the following volumes: 200 $\mu\text{l}$  Methyltransferase Reaction Buffer Concentrate, 22 $\mu\text{l}$  substrate and 1778 $\mu\text{l}$  1X Transferase Assay Buffer. Diluted Reaction Mix should be kept on ice and used within 8 hours of preparation. Any unused 1X Reaction Mix should be discarded.

#### 4. Positive Control

In the absence of enzyme, the reaction buffer converts the positive control into the reaction's end product, and yields signal when detection solution is applied. This interaction confirms the activity of the kit components. It should not be used to calculate the concentration of methyltransferase activity in samples.

Prepare enough Positive Control to test in duplicate. Use the following formula to calculate the volume of Positive Control required. A minimum of 2 wells of the positive control are recommended per assay.

A. Total volume required

$$[\text{Total number of wells needed}] \times 25 \mu\text{l} = \text{_____} \mu\text{l}$$

B. Volume of Positive Control required

$$[\text{Total volume required (from A. above)}] \times 0.1 = \text{_____} \mu\text{l}$$

C. Volume of 1X Transferase Assay Buffer required

$$[\text{Total volume required (from A. above)}] \times 0.90 = \text{_____} \mu\text{l}$$

Prepare Positive Control on ice by combining the appropriate reagent volumes calculated in B and C above. For example, to prepare 200 $\mu$ l of Positive Control, combine the following volumes: 20 $\mu$ l of Positive Control and 180 $\mu$ l of 1X Transferase Assay Buffer. Diluted Positive Control should be kept on ice and used within 8 hours of preparation. Any unused 1X Positive Control should be discarded.

## ASSAY PROCEDURE

Refer to the Assay Layout Sheet to determine the number of wells to be used.

Cover unused wells tightly with a plate sealer. **DO NOT REUSE WELLS!**

1. Pipet 25 $\mu$ l of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 25 $\mu$ l of methyltransferase into the bottom of the appropriate wells.
3. Pipet 100 $\mu$ l 1X Detection Solution into each well.
4. Pipet 25 $\mu$ l of 1X Reaction Mix (containing SAM dilutions) into each well.
5. Cover plate with foil plate sealer. Incubate for 20 min (for end point format) shaking\* at room temperature.
6. Remove plate from shaker.
7. Pipet 25 $\mu$ l of 1X Positive Control into the bottom of the appropriate wells.
8. Pipet 100 $\mu$ l Detection Solution into Positive Control wells.
9. Pipet 25 $\mu$ l of 1X Reaction Mix into Positive Control wells.
10. Cover plate with foil plate sealer. Incubate for 10 min shaking\*.
11. Pipet 50 $\mu$ l of stop solution\*\* into each well.
12. Read fluorescence at 380ex/520em.

\*Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.



All standards and samples should be run in duplicate.



Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample and reagent.



Pipet the reagents to the sides of the wells to avoid possible contamination.

\*\*Possible stop solutions (choice of): isopropyl alcohol (ice cold), 1mM NEM, or 5mM Adenine 9 $\beta$  Arabinoside. As a suggested starting point, we recommend using NEM at a concentration that, when applied to the well, will result in a final 1mM concentrations. For example, if adding 50 $\mu$ L of stop solution to a 150 $\mu$ L mixture in the well, use a 4mM NEM solution.

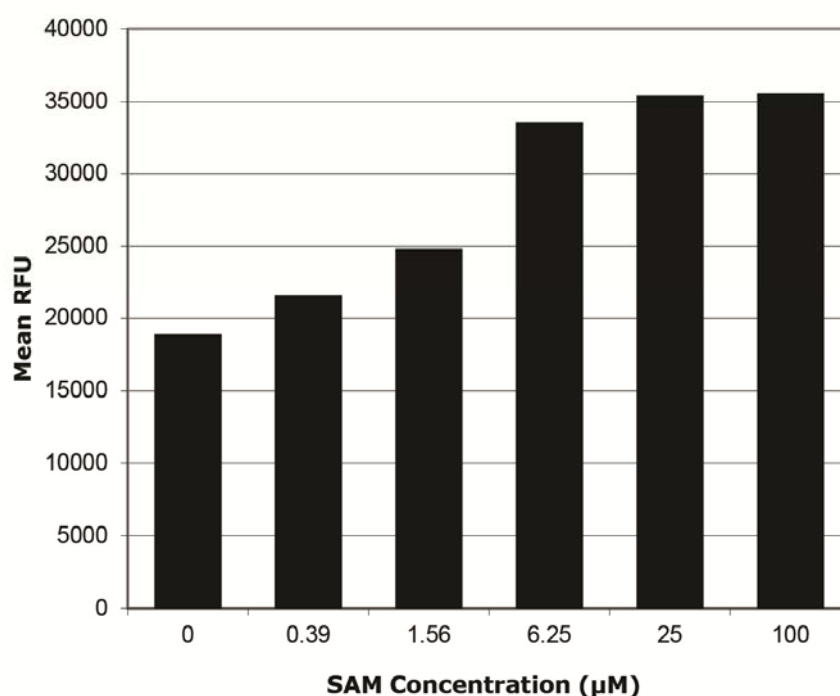
## EXAMPLE OF END POINT ASSAY RESULTS

1. Plot the mean of the duplicate relative fluorescence units (RFU) at 380ex/520em versus SAM concentration.
2. Calculate the signal to noise ratio :

$$\frac{\text{mean RFU for enzyme dilution}}{\text{mean RFU blank.}}$$

SAM was titrated in the assay using 0.5 $\mu$ M of the histone methyltransferase, SET7/9, and 15 $\mu$ M of the peptide substrate TAF-10. Serial dilutions of SAM were prepared in Reaction Mix containing TAF-10. Average relative fluorescence was plotted against SAM concentration to generate the following graph. This is for illustration purposes only. The investigator must titrate SAM in the assay with their enzyme/substrate system of choice.

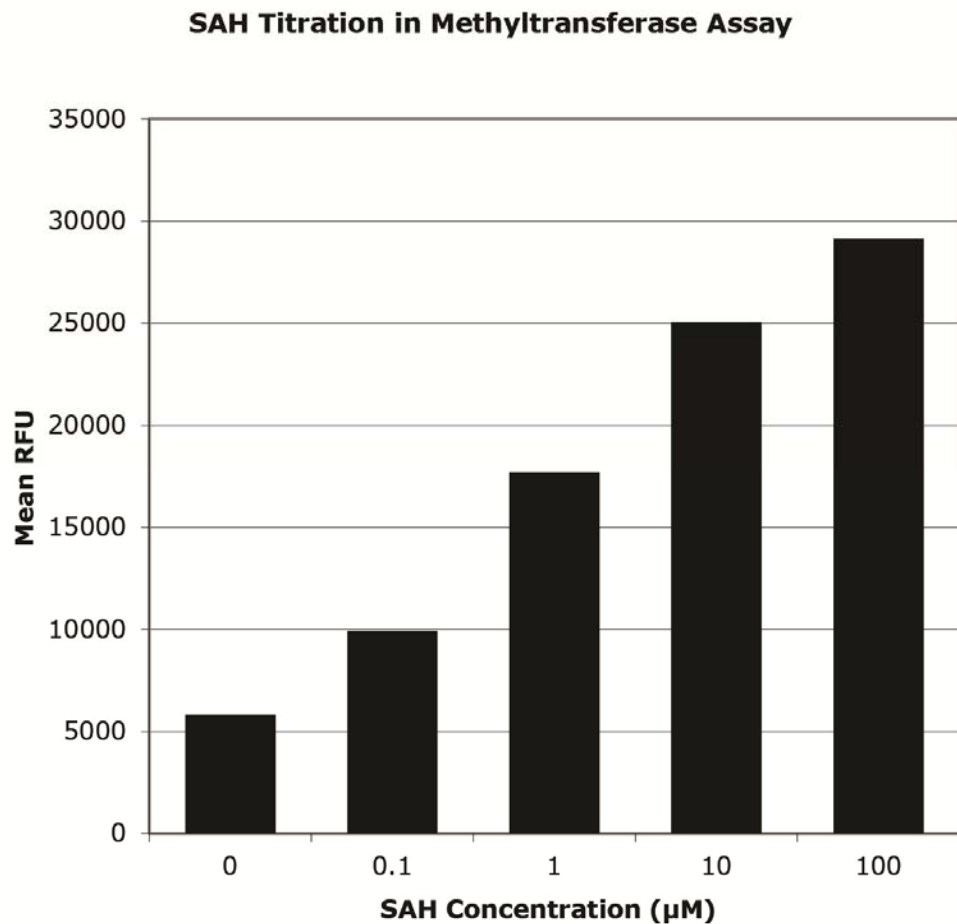
**SAM Titration in Methyltransferase Assay**



Based on this titration data, a SAM concentration of at least 25 $\mu$ M is needed to achieve enzyme saturation. Also, the maximum signal is within the detection range of the plate reader, with a signal to noise ratio sufficient for easy detection of altered enzyme activity.

### EXAMPLE OF SAH TITRATION RESULTS

The positive control may be serially diluted and run in the assay in place of the methyltransferase to estimate the concentration of end product needed to produce a given signal. Mean relative fluorescence was plotted against SAH concentration to generate this graph. This graph is for illustration purposes only.



The sensitivity of the assay can be described as the amount of methyltransferase needed to generate enough SAH to produce a signal to noise ratio sufficient for easy detection of enzyme activity.

## TYPICAL INHIBITION ASSAY PROCEDURE

It may be necessary to determine the optimal concentrations of methyltransferase/acceptor substrate to use prior to screening candidate compounds. An end-point assay using the empirically determined concentration of SAM is recommended. Refer to the Assay Layout Sheet to determine the number of wells to be used. DO NOT REUSE WELLS!

1. Pipet 25µl of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 10µl of 1X Transferase Assay Buffer into the zero wells.
3. Pipet 10µl of inhibitor dilution into the bottom of the appropriate wells.
4. Pipet 15µl of methyltransferase at chosen working concentration into appropriate wells.
5. Cover plate with foil plate sealer. Incubate for 10 min at room temperature without shaking.
6. Pipet 25µl of 1X Reaction Mix into each well.
7. Pipet 100µl of 1X Detection Solution into each well.
8. Cover plate with foil plate sealer. Incubate for 30 min, shaking\* at room temperature.
9. Pipet 50µl of stop solution\*\* into each well.
10. Read fluorescence at 380ex/520em.

\* Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.

\*\* Possible stop solutions (choice of): isopropyl alcohol (ice cold), 1mM NEM, or 5mM Adenine 9β Arabinoside. As a suggested starting point, we recommend using NEM at a concentration that, when applied to the well, will result in a final 1mM concentrations. For example, if adding 50µL of stop solution to a 150µL mixture in the well, use a 4 mM NEM solution.

## CALCULATION OF RESULTS

Several options are available for the calculation of the inhibition of methyltransferase. We recommend that the data be handled by a software package utilizing a suitable curve fitting program to determine the percent inhibition. If data reduction software is not readily available, the data can be calculated as follows:

1. Calculate the mean net RFU for each sample by subtracting the mean blank RFU from the mean RFU for the samples:

$$\text{Mean Net RFU} = \text{Mean Sample RFU} - \text{Mean Blank RFU}$$

2. Percent inhibition should be calculated using the following formula for each inhibitor dilution:

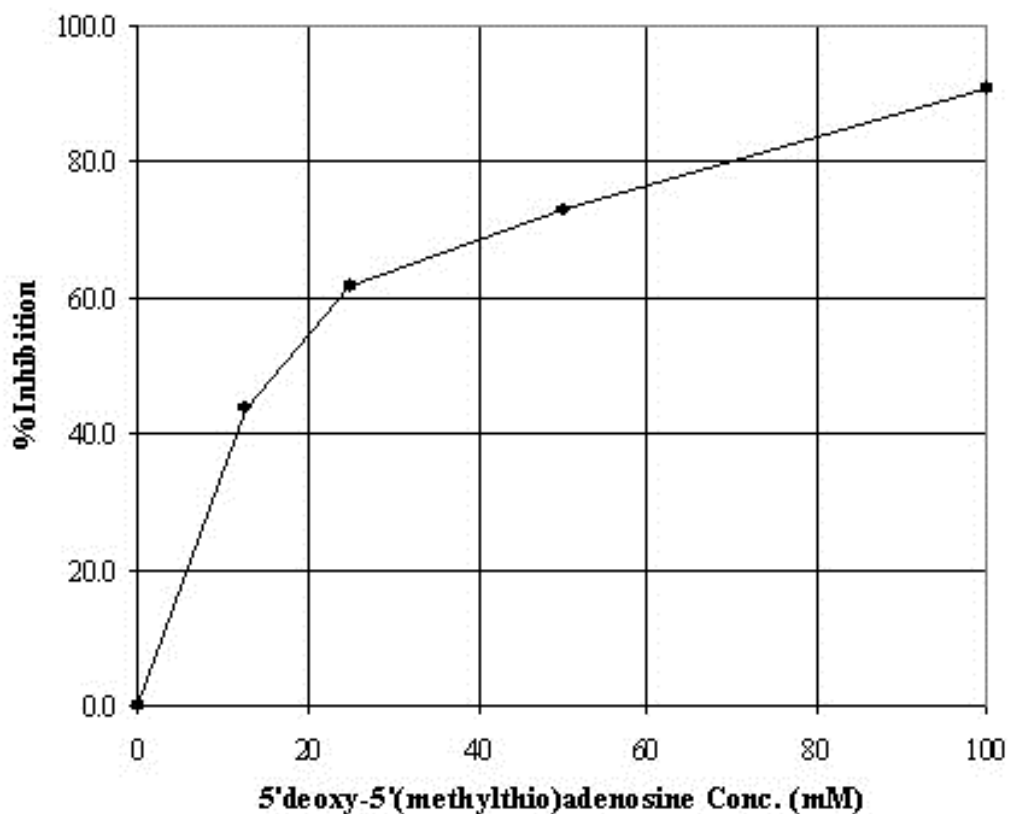
Percent Inhibition =

$$\frac{(\text{Mean Net Zero (non-inhibited enzyme) RFU} - \text{Mean Net Inhibited enzyme RFU}) \times 100}{\text{Mean Net Zero (non-inhibited enzyme) RFU}}$$

## TYPICAL INHIBITION ASSAY RESULTS

Using SET7/9 and TAF-10 as an enzyme substrate system, percent inhibition for dilutions of 5'deoxy-5'(methylthio)-adenosine was tested. The results shown below are for illustration only and should not be used to calculate results from another assay.

Dilution	Inhib. Conc. (mM)	Mean RFU	Mean Net RFU	% Inhibition
1	100mM	12828	1135	90.7
2	50mM	15030	3337	72.7
3	25mM	16411	4718	61.4
4	12.5mM	18575	6882	43.7
0	0mM	23910	12217	0
blank	-----	11693	0	-----



## TYPICAL ENZYME INTERFERENCE ASSAY PROCEDURE

1. Pipet 25 $\mu$ l of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 10 $\mu$ l of 1X Transferase Assay Buffer into the zero wells.
3. Pipet 10 $\mu$ l of colored compound dilution into appropriate wells.
4. Pipet 15 $\mu$ l of 1X Positive Control into the zero wells, and wells containing colored compound dilutions.
5. Pipet 25 $\mu$ l of 1X unspiked reaction mix into each well.
6. Pipet 100 $\mu$ l 1X Detection Solution into each well.
7. Cover plate with foil plate sealer, incubate for 10 min at room temperature without shaking.
8. Pipet 50 $\mu$ l of stop solution\*\*into each well.
9. Read fluorescence at 380ex/520em.

\*\*Possible stop solutions (choice of): isopropyl alcohol (ice cold), 1mM NEM, or 5mM Adenine 9 $\beta$  Arabinoside. As a suggested starting point, we recommend using NEM at a concentration that, when applied to the well, will result in a final 1mM concentrations. For example, if adding 50 $\mu$ L of stop solution to a 150 $\mu$ L mixture in the well, use a 4 mM NEM solution.

To determine whether or not colored compounds will interfere with the assay, calculate and compare the signal to noise ratio of the colored compound dilutions to the signal to noise ratio of the zero wells.



## INTERFERING SUBSTANCES

The following solvents were tested for interference with the fluorescent signal generated in the assay. The table lists the percentage of signal in the presence of interferant relative to the zero for each solvent.

% Interferant	DMSO	DMF	Acetonitrile
12.5	108	68	49
6.25	107	82	71
3.12	107	88	86
1.56	112	98	96
0.78	114	104	95
0.39	115	109	100
0	100	100	100

\*Percent interferant is relative to a 50 $\mu$ l total reaction volume.

Diluents containing bovine serum albumin (BSA), or other Thiol containing reagents, should be treated with N-Ethylmaleimide (NEM) prior to use in the assay. We recommend reacting 10% BSA with 1mM NEM for 1 hour at room temperature, however this procedure should be optimized for each reagent.

**REFERENCES**

1. Walsh, C.T. Protein Methylation in Posttranslational Modifications of Proteins, Expanding Nature's Inventory. Roberts and Co. Pub.; Englewood, USA.
2. Hayashi, K. et. al. A Histone H3 Methyltransferase Controls Epigenetic Events Required for Meiotic Prophase. *Nature* (2005) 438(17):374-378.
3. Mavrodi, D.V. et. al. Functional Analysis of Genes for Biosynthesis of Pyocyanin and Phenazine-1-Carboxamide from *Pseudomonas aeruginosa* PAO1. *J. Bact.* (2001) 183(21):6454-6465.
4. Chubb, J.R. et. al. Developmental Timing in *Dictyostelium* is Regulated by the SET1 Histone Methyltransferase. *Devel. Bio.* (2006) 292:519-532.
5. Miao, F. et. al. Coactivator-Associated Arginine methyltransferase-1 Enhances Nuclear Factor- $\kappa$ B-Mediated Gene Transcription Through Methylation of Histone H3 at Arginine 17. *Mol. Endocrin.* (2006) 20(7):1562-1573.
6. Ehrenhofer-Murray, A.E. Chromatin Dynamics at DNA Replication, Transcription and Repair. *Eur. J. Biochem.* (2004) 271:2335-2349.
7. Kouskouti, A., et. al. Gene-Specific Modulation of TAF10 Function by SET9-Mediated Methylation. *Mol. Cell* (2004) 14:175-182.
8. Chulkov, S. et. al. Regulation of p53 Activity Through Lysine Methylation. *Nature* (2004) publ on-line (doi:10.1038/nature03117).
9. Fuks, F. et. al; The DNA Methyltransferases Associate with HP1 and the SUV399H1 Histone Methyltransferase. *Nucl. Acids Res.* (2003) 31(9):2305-2312
10. Chaing, P.K., et. al. S-Adenosylmethionine and Methylation. *FASEB J.* (1996) 10:471-480.
11. Trievel, R.C. Structure and Function of Histone Methyltransferases. *Critical Review<sup>TM</sup> in Eukaryotic Gene Expression* (2004) 14(3):147-169.
12. Robertson, K.D. DNA Methylation and Human Disease. *Nature Rev.* (2005) DNA Methylation Collection:7-20.
13. Wei, S.H. et.al. Methylation Microarray Analysis of Late-Stage Ovarian Carcinomas Distinguishes Progression-free Survival in Patients and Identifies Candidate Epigenetic Markers. *Clin. Cancer Res.* (2002) 8:2246-2252.
14. Castro, R. et. al. Increased Homocysteine and S-Adenosylhomocysteine Concentrations and DNA Hypomethylation in Vascular Disease. *Clin. Chem.* (2003) 49(8):1292-1296.



# Product Manual

## NOTES



Check our website for additional protocols, technical notes and FAQs.

#### **GLOBAL HEADQUARTERS**

Enzo Life Sciences Inc.  
10 Executive Boulevard  
Farmingdale, NY 11735  
Toll-Free: 1.800.942.0430  
Phone: 631.694.7070  
Fax: 631.694.7501  
[info-usa@enzolifesciences.com](mailto:info-usa@enzolifesciences.com)

#### **EUROPE/ASIA**

Enzo Life Sciences (ELS) AG  
Industriestrasse 17  
CH-4415 Lausen  
Switzerland  
Phone: +41/0 61 926 89 89  
Fax: +41/0 61 926 89 79  
[info-ch@enzolifesciences.com](mailto:info-ch@enzolifesciences.com)

For local distributors and detailed product information visit us online:  
[www.enzolifesciences.com](http://www.enzolifesciences.com)