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-907-025

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

USE FOR RESEARCH PURPOSES ONLY

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Product Manual

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.

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INTRODUCTION

The Methyltransferase activity kit is a complete kit for the screening of candidate compounds that may alter normal methyltransferase activity. 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.[1-9] 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-adenosyl-L-Methionine as a universal donor.[10] Part of the acceptor diversity of this enzyme family relates to the flexible structural folds that bind these molecules in proximity of the donor.[11] The side-chains of lysine, arginine, glutamate, glutamine, asparagines, and isoprenylated residues serve as methylation sites in proteins like histones.[1] Changes in methylation patterns have been tightly linked to disease states such as cancer and vascular disease.[12-14]

PRINCIPLE

1. Enzyme, reaction mix spiked with acceptor substrate, and detection solution are added to wells of a black 96-well plate. The plate is then incubated.
2. Ice cold isopropyl alcohol is added to stop the reaction.
3. The plate is transferred to a plate reader and fluorescence is measured at 380ex/520em.

Methyltransferase

Acceptor Substrate+Reaction Buffer+Detection Solution–>Fluorescence

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. **Methyltransferase Reaction Buffer Concentrate**

   0.3ml, Catalog No. 80-1649

   A 10X concentrated buffer containing S-adenosyl-L-methionine.

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.

6. **Foil Plate Sealer**

   3 each, Catalog No. 10-3126

7. **Methyltransferase Assay Layout Sheet**

   1 each, Catalog No. 30-0241

Reagents
require
separate
storage
conditions.

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-adenosyl-L-methionine 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 9ß Arabinoside.

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

[Total number of wells needed + 6] x 100µl   = _____µl

B. Volume of Transferase Detection Solution Concentrate required

[Total volume required (from A. above)] x 0.01 = _____µl

C. Volume of 1X Transferase Assay Buffer required

[Total volume required (from A. above)] x 0.99 = _____µl

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: 20µl of the supplied Transferase Detection Solution Concentrate and 1980µl 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

[Total number of wells needed + 6] x 25µl   = _____µl

B. Volume of Methyltransferase Reaction Buffer Concentrate required

[Total volume required (from A. above)] x 0.1 = _____µl

C. Volume of acceptor substrate required

[Determined empirically based on enzyme used]= _____µl

D. Volume of 1X Transferase Assay Buffer required

[Total volume required (from A. above)] x 0.90 – [volume of substrate required] = _____µl

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 22µl of acceptor substrate, combine the following volumes: 200µl Methyltransferase Reaction Buffer Concentrate, 22µl substrate and 1778µl 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

[Total number of wells needed] x 25µl = _____µl

B. Volume of Positive Control required

[Total volume required (from A. above)] x 0.1 = _____µl

C. Volume of 1X Transferase Assay Buffer required

[Total volume required (from A. above)] x 0.90 _____µl

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.

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

Prepare Positive Control on ice by combining the appropriate reagent volumes calculated in B and C above. For example, to prepare 200µl of Positive Control, combine the following volumes: 20µl of Positive Control and 180µl 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.

SAMPLE HANDLING

This assay is suitable for use with all S-adenosyl-L-methionine dependent methyltransferases. It is necessary to titrate 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.

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

It is recommended that an end-point assay be performed to determine the optimal concentration of enzyme/substrate to use prior to screening candidate compounds. Make serial dilutions of the methyltransferase of interest in the assay buffer. Initial concentrations of 100nM are recommended. A kinetic assay format is also an available option.

The positive control provided may also be used to test colored compounds for interference in the assay. A suitable protocol follows.

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.

END POINT / KINETIC 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 25µl of 1X Transferase Assay Buffer into the blank wells.

2. Pipet 25µl of methyltransferase dilutions into the bottom of the appropriate wells.

3. Pipet 25µl of 1X Reaction Mix into each well.

4. Pipet 100µl 1X Detection Solution into each well.

5. Cover plate with foil plate sealer. Incubate for 20 min (for end point format) shaking* at room temperature.

For Kinetic measurements, incubate identical reaction wells for the desired periods of time. The duplicate wells for each time point can be stopped as in step #10 below.

6. Pipet 25µl of 1X Positive Control into the bottom of the appropriate wells.

7. Pipet 25µl of 1X Reaction Mix into Positive Control wells.

8. Pipet 100µl Detection Solution into Positive Control wells.

9. Cover plate with foil plate sealer. Incubate for 10 min.

10. Pipet 50µl of stop solution** into each well.

11. 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 9ß 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 4mM NEM solution.

EXAMPLE OF END POINT ASSAY RESULTS

1. Plot the mean of the duplicate relative fluorescence units (RFU) at 380ex/520em versus Enzyme concentration.

2. Calculate the signal to noise ratio :

   mean RFU for enzyme dilution

   mean RFU blank.

An engineered portion of the human lysine specific histone methyltransferase, SET7/9 was titered in the assay using 15µM of the peptide substrate TAF-10. Serial dilutions of the enzyme were prepared in Transferase Assay Buffer. Average relative fluorescence was plotted against SET7/9 concentration to generate the following graph. This is for illustration purposes only. The investigator must titrate their own enzyme substrate system in the assay.
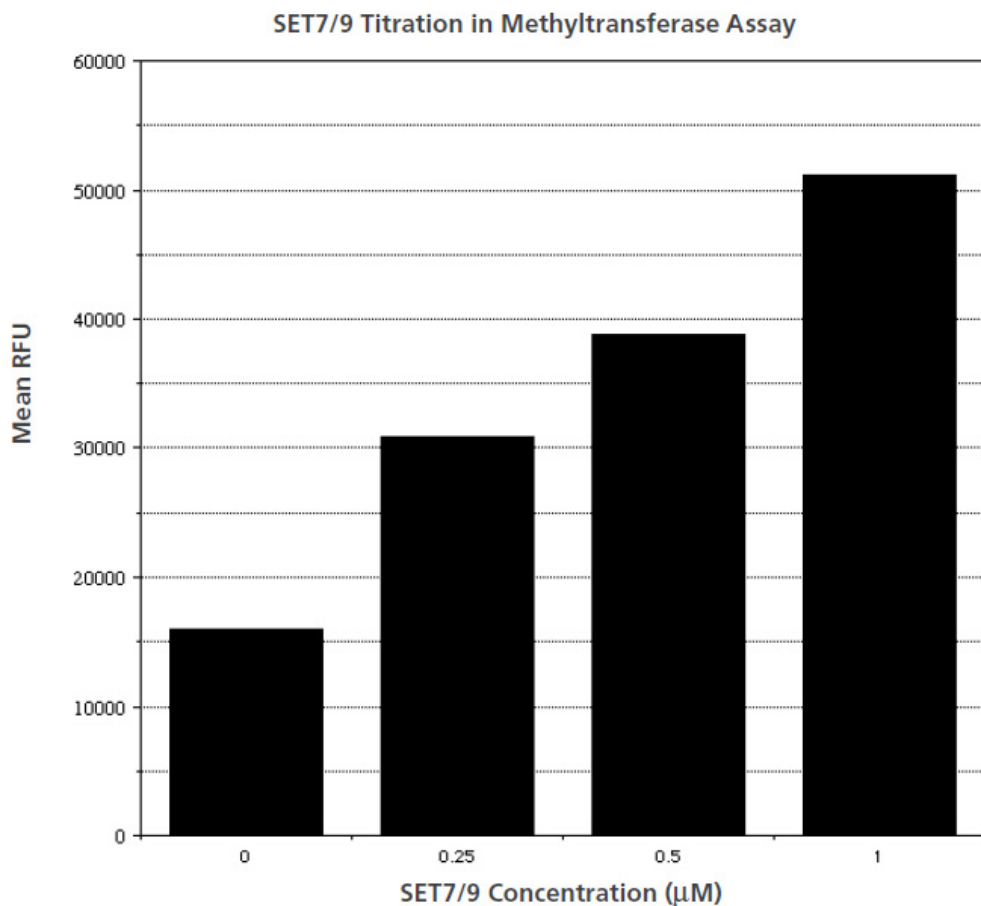
Based on this titration data, the methyltransferase concentration of 0.5µM produces a maximum signal within the detection range of the plate reader, with a signal to noise ratio sufficient for easy detection of altered enzyme activity.

EXAMPLE OF KINETIC ASSAY RESULTS

0.5µM SET7/9 was tested with 15µM TAF-10 substrate in the kinetic assay format. Mean relative fluorescence was plotted against the stop time interval to generate this graph. This graph is for illustration purposes only. The kinetic assay must be optimized by the investigator, with their enzyme / substrate system.
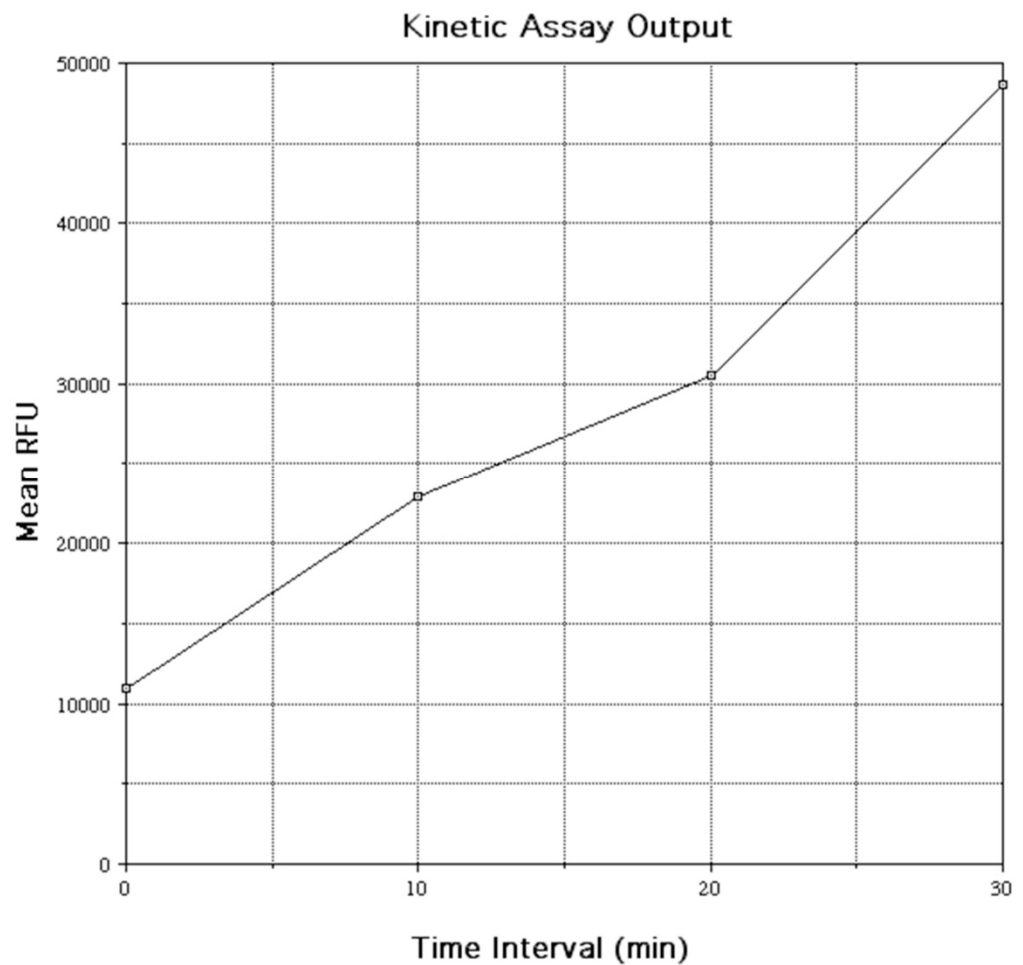
EXAMPLE OF SAH TITRATION RESULTS

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.

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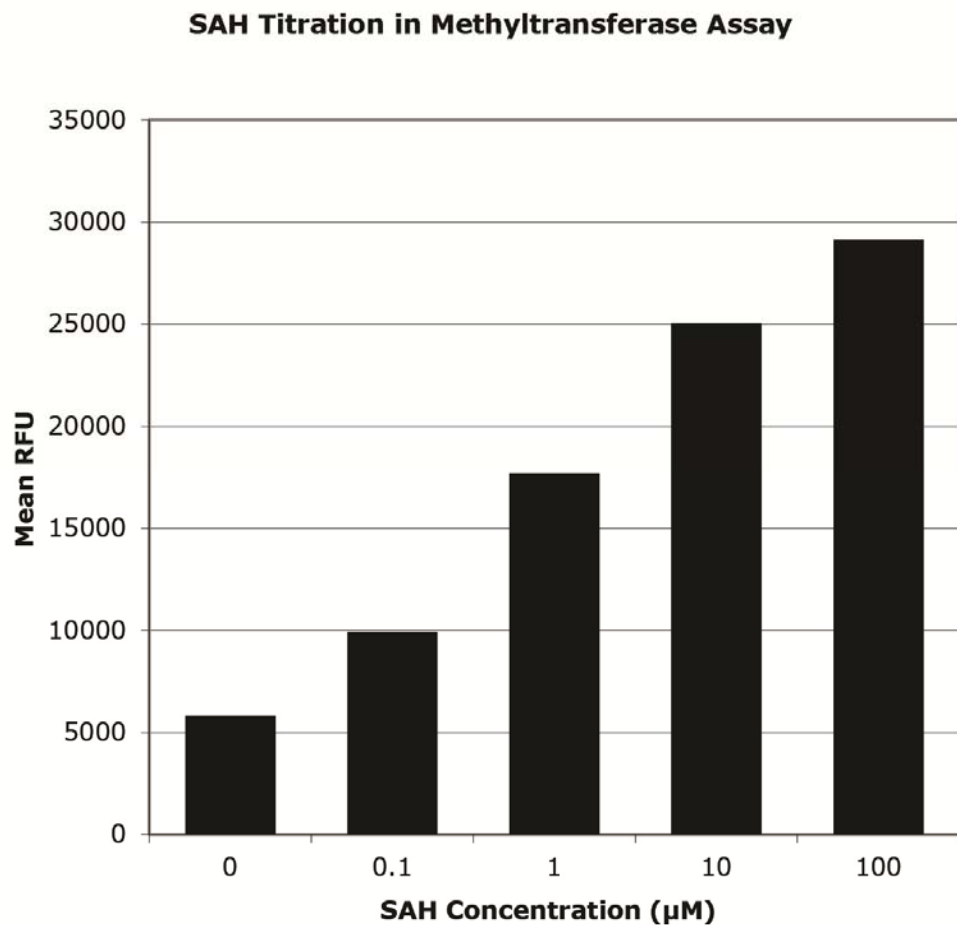
The sensitivity of the assay can be described as the amount of methyltransferase needed to generate enough SAH to produce a signal to noise ratio sufficient for easy detection of enzyme activity.

TYPICAL INHIBITION ASSAY PROCEDURE

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 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 9ß 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 4mM 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:

   Mean Net RFU = Mean Sample RFU - 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	------
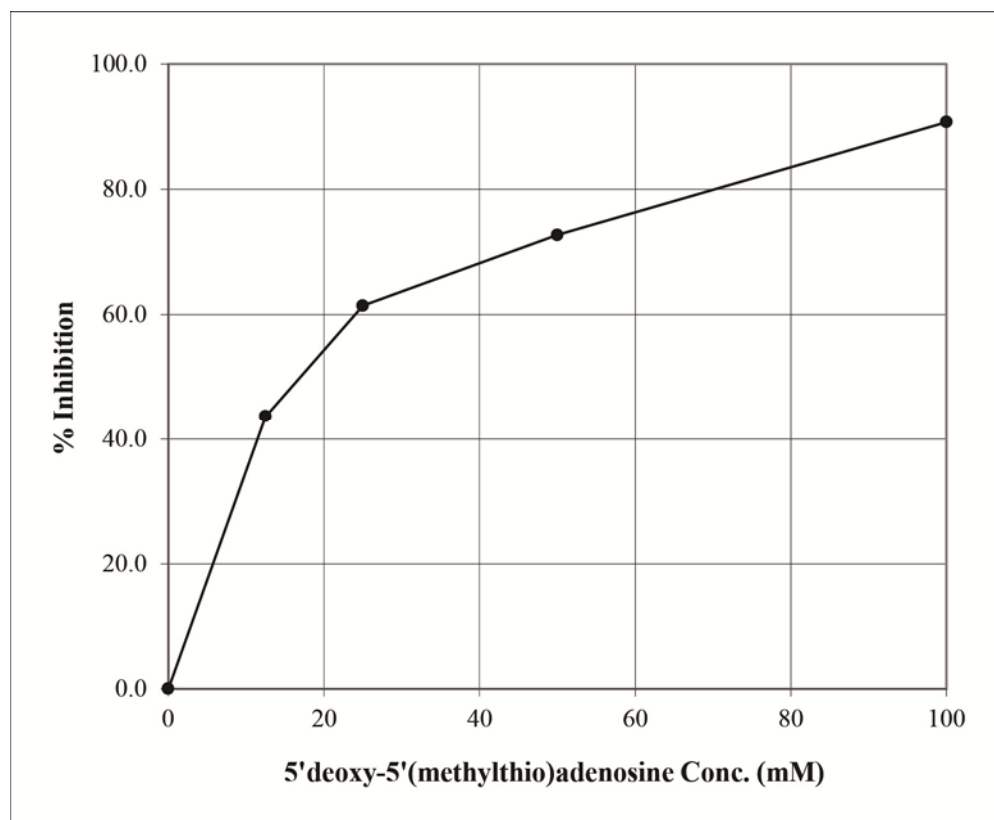
TYPICAL ENZYME INTERFERENCE ASSAY PROCEDURE

1. Pipet 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 colored compound dilution into appropriate wells
4. Pipet 15μl of 1X Positive Control into the zero wells, and wells containing colored compound dilutions.
5. Pipet 25μl of 1X unspiked reaction mix into each well.
6. Pipet 100μl 1X Detection Solution into each well.
7. Cover plate with foil plate sealer, incubate for 10 min at room temperature without shaking.
8. Pipet 50μl 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 9ß 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 4mM 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 50µl 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

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