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A Geno Technology, Inc. (USA) brand name

SAM510: SAM Methyltransferase Assay

A Non Radioactive Colorimetric Continuous Enzyme Assay

(Cat. # 786-430)

INTRODUCTION

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. Aberrant levels of SAM have been linked to many abnormalities, including Alzheimer's, depression, Parkinson's, multiple sclerosis, liver failure and cancer (2).

The SAM510: SAM Methyltransferase Assay is a continuous enzyme coupled assay that can continuously monitor SAM-dependent methyltransferases (3) without the use of radioactive labels or endpoint measurements.

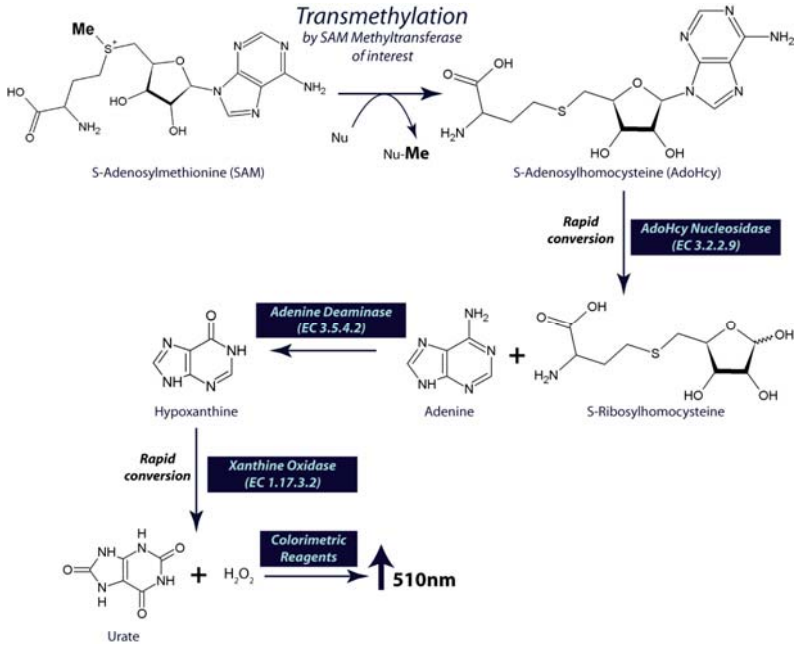

Figure 1: SAM510: SAM Methyltransferase AssayScheme

Figure 1 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 the included 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 hydrogen peroxide is measured with a colorimetric reagent, 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS), by an increase in absorbance at 510nm. The kit is supplied with enough reagents for 100 microwell assays.

The assay is supplied with AdoHcy as a positive control. The assay can be adapted to be used with any purified SAM dependent methyltransferase or a purified enzyme that produces 5-adenosylhomocysteine or 5'-methylthioadenosine, due to the specificity of AdoHcy nucleosidase.

Patent Pending, available under a licensing agreement with Washington State University, Pullman WA

ITEM(S) SUPPLIED (Cat. # 786-430)

Description	Size
SAM Methyltransferase Assay Buffer	20ml
SAM Methyltransferase Assay Buffer Additive	0.2ml
SAM Enzyme Mix	3 x 300µl
SAM Colorimetric Mix	1
Positive Control: Adenosylhomocysteine [1mM]	0.2ml
S-Adenosylmethionine	3 vials
HCl Assay Reagent [20mM]	1ml

STORAGE CONDITIONS

The kit is shipped on dry ice. Upon arrival, store the kit at -70°C. The kit components are stable for up to 1 year, when stored and used as recommended.

ADDITIONAL ITEMS REQUIRED

- Purified SAM Methyltransferase to be tested
- Appropriate methyltransferase acceptor substrate
- Plate reader suitable for half volume (115µl) to measure between 500-520nm absorbances

IMPORTANT INFORMATION

- The final volume of the assay is 115µl in all the wells
- All reagents, except the enzymes, must be equilibrated to room temperature before use
- We recommend assaying samples in triplicate.
- Assay is performed at 37°C

PREPARATION BEFORE USE

1. Thaw SAM Methyltransferase Assay Buffer and SAM Methyltransferase Assay Buffer Additive solution at room temperature. Add the entire volume of SAM Methyltransferase Assay Buffer Additive into the SAM Methyltransferase Assay Buffer and mix it thoroughly. Store SAM Methyltransferase Assay Buffer at room temperature, do not freeze after addition of Additive.
2. **Positive Control: Adenosylhomocysteine**: The vial contains 200µl of a 1mM solution of adenosylhomocysteine (AdoHcy). Thaw the vial on ice.
3. **SAM Enzyme Mix**, supplied in 300µl vials. Each vial is suitable for 36 assays. Thaw on ice only the number of vials you require for your assay. We do not recommend repeated freeze/thawing of the SAM Enzyme Mix.
4. **S-Adenosylmethionine (SAM)**, supplied lyophilized. Reconstitute the contents of the vial with 100µl HCl Assay Reagent [20mM] to yield 6.9mM SAM. Each vial is suitable for 36 assays. We do not recommend repeated freeze/thawing of the S-Adenosylmethionine solution.
5. **SAM Colorimetric Mix**, supplied lyophilized. Add 1ml SAM Methyltransferase Assay Buffer with additive to the vial of SAM Colorimetric Mix. Wait 5 minutes and then vortex until dissolved. If not using all of the SAM Colorimetric Mix, we recommend aliquoted into smaller vials and storing at -70°C.
6. **Sample Preparation:** Prepare your test sample, containing the purified SAM dependent methyltransferase to be assayed, according to your own standard protocol. Avoid the use of reducing agents and metal chelators as these have an inhibitory effect on the reaction. If these reagents are present, we recommend using our Tube-O-DIALYZER™ micro devices to dialyze the sample against 0.1M Tris-HCl, pH8.0.
7. Prepare the specific substrate for the methyltransferase to be assayed using the SAM Methyltransferase Assay Buffer or the buffer of your own choice. Avoid the use of reducing agents and metal chelators as these have an inhibitory effect on the reaction.

PROTOCOL

NOTE: *The positive control supplied is a control for the SAM Methyltransferase Assay. Some acceptor substrates, inhibitors, activators or buffer components may interfere with the assay. We highly recommend testing the compatibility of these reagents with the SAM Methyltransferase Assay using the positive control, by substituting in suspected non compatible reagent into the positive control reactions. It is necessary to titrate each enzyme/ substrate system in the assay to determine optimal conditions.*

NOTE: *The deaminase in the SAM methyltransferase assay requires the presence of divalent metal ions, present in the SAM Methyltransferase Assay Buffer. The presence of chelators, such as EDTA, will deplete divalent metal ions and inhibit the deaminase enzyme. If EDTA is required then supplement additional manganese ions into the reaction.*

NOTE: *Reducing agents, including DTT, β-mercaptoethanol and TCEP, have an inhibitory effect on the assay. If present, we recommend dialysis against 0.1M Tris-HCl, pH8.0.*

1. Equilibrate the SAM Methyltransferase Assay Buffer + Additive to 37°C.
   NOTE: *The SAM Buffer + Additive must be at 37°C prior to performing the assay. Failure to prewarm will result in artifactual results.*
2. Aliquot a total volume of 5µl of your SAM methyltransferase samples to at least two wells of a 96 well plate. Use the SAM Methyltransferase Assay Buffer or 0.1M Tris, pH8.0 as a diluent. We recommend performing the reactions and controls in at least duplicate.
   a. **For the background control**, aliquot 5µl SAM Methyltransferase Assay Buffer into each background control well. We recommend performing the reactions in duplicate.
   b. **For the positive control**, add 5µl Positive Control and 10µl SAM Methyltransferase Assay Buffer to each positive control well. We recommend performing the reactions in duplicate.
3. Add 10µl the appropriate acceptor substrate to the sample and background control wells, using SAM Methyltransferase Assay Buffer or 0.1M Tris, pH8.0 as a diluent.
   NOTE: *If assaying inhibitors or activators, adjust the acceptor substrate concentration so that the substrate and activators or inhibitors are added in a final volume of 10µl.*

4. Immediately prior to use and in a suitable tube, prepare the SAM Methyltransferase Assay Master Mix according to the table below:

Reagent	**36 wells**	**72 wells**	**100 wells**
SAM Methyltransferase Assay Buffer + Additive	3ml	6ml	9ml
SAM Enzyme Mix	1 vial/ 300µl	2 vials/ 600µl	3 vials/ 900µl
SAM Colorimetric Mix	200µl	400µl	600µl
S-Adenosylmethionine	1 vial/ 100µl	2 vials/ 200µl	3 vials/ 300µl

5. Immediately initiate the reaction by adding 100µl SAM Methyltransferase Master Mix to the wells. Immediately, zero the wells and begin measuring the absorbances at 510nm collecting data every 10-30 seconds at 37°C until the increasing absorbances plateau (approx. 15-30mins).

ANALYSIS

A. Calculate the average absorbance of each sample.

B. Calculate the change in absorbance (ΔAbs) per minute, by:

   i. Either, plot the absorbances against time to obtain the slope (rate) of the linear portion of the curve. See figure 2 for a plot with the AdoHcy positive control and figure 3 for an example using humane lysine specific histone Methyltransferase, SET7/9). Subtract the background control from your samples and positive control and then plot a curve of time (minutes) against absorbance. The rate of the enzyme activity can be characterized by the absorbance increase per minute.

   ii. Or, calculate the change in absorbance between two points on the linear portion of the curve using the following equation:

$$\Delta\text{Abs/min} = \frac{(\text{Abs at Time 2}) - (\text{Abs at Time 1})}{\text{Time 2 (min)} - \text{Time 1 (min)}}$$

C. Calculate the rate of ΔAbs/min for the background control wells and subtract this rate from the sample well rate.

D. Calculate Methyltransferase Activity: Use the following equation to calculate the Methyltransferase activity:

$$\text{Methyltransferase Activity (µmol/min/ml)} = \frac{\Delta\text{Abs/min}}{15.0\text{mM}^{-1}} \times \frac{0.115\text{ml}}{0.005\text{ml}} \times \text{Sample Dilution}$$

The rate of the reaction is determined using the 3 ,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) extinction coefficient 15.0mM^{-1}*. One unit of Methyltransferase will transfer 1.0µmol of a methyl group per minute at 37°C.

**NOTE: The actual extinction coefficient of 3 ,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) is $26.0\text{mM}^{-1}\text{cm}^{-1}$. This value has been adjusted for the pathlength of the solution in the well (0.577cm).*

E. If activators or inhibitors were assayed, determine the percent activation/ inhibition for each sample as follows:

$$\%\ \text{Inhibition} = \frac{(\text{Activity untreated sample}) - (\text{Activity Inhibitor Treated})}{\text{Activity untreated sample}} \times 100$$

$$\%\ \text{Activation} = \frac{(\text{Activity Activator Treated}) - (\text{Activity untreated sample})}{\text{Activity untreated sample}} \times 100$$

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ASSAY RANGE

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

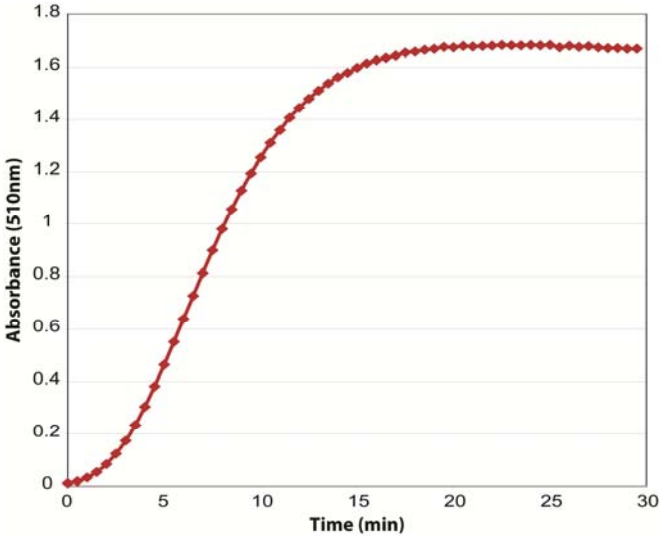


Figure 2: SAM510 Assay quantitatively assays the Positive Control: 50µM Adenosylhomocysteine (AdoHcy).

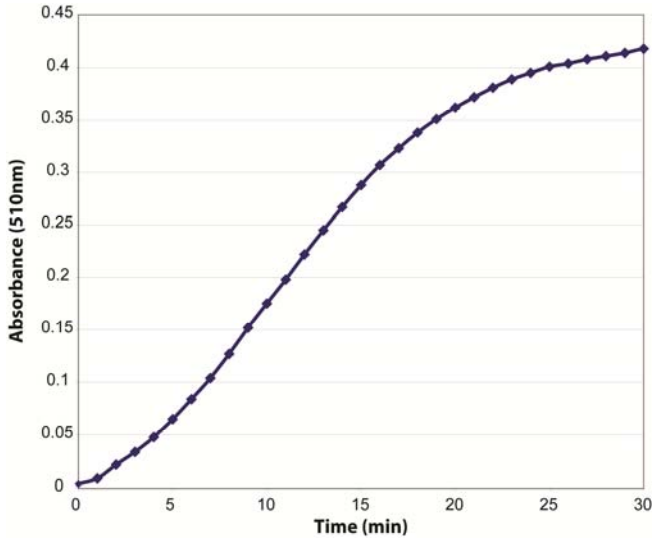


Figure 3: Human lysine specific histone Methyltransferase SET7/9 assayed with 20µM TAF-10 as the acceptor substrate.

TROUBLESHOOTING

Issue	Possible Cause	Recommended Solution
Erratic values; dispersion duplicates/ triplicates	• Poor pipetting technique • Air Bubbles in well	• Use a repeating pipette and do not eject solution too vigorously • Gently tap plate to dislodge air bubbles
No absorbance detected above background control	• Sample too dilute or acceptor substrate not added • Acceptor substrate interferes with assay	• Re-assay using a lower dilution and ensure acceptor substrate is added • Add the acceptor substrate in with the positive control to test compatibility
Color development was too fast	• Too much enzyme in well	• Dilute your samples in assay buffer or 0.1M Tris, pH8.0
No inhibition or activation was seen with added compounds	• The compound concentration was too low • Compound is not an inhibitor or activator	• Increase compound concentration and re-assay

REFERENCES

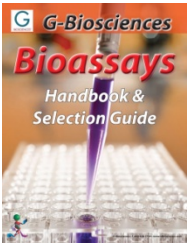
1. Cheng, X. and Blumenthal, R.M. (1999) S-Adenosylmethionine Dependent Methyltransferases: Structures and Functions, World Scientific, Singapore.
2. Schubert, H.L. et al. (2003) Trends Biochem. Sci 28: 329-335.
3. Dorgan, K.M. et al. (2006) Anal. Biochem. 350:249-255.

CITATIONS

1. Kumar, A. et al (2011) J. Biol. Chem. 286:19652-19661

RELATED PRODUCTS

Download our Bioassays Handbook.



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