User Manual

SAM510: SAM Methyltransferase Assay

A Non-Radioactive Colorimetric Continuous Enzyme Assay

CBA096

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Product Overview

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.

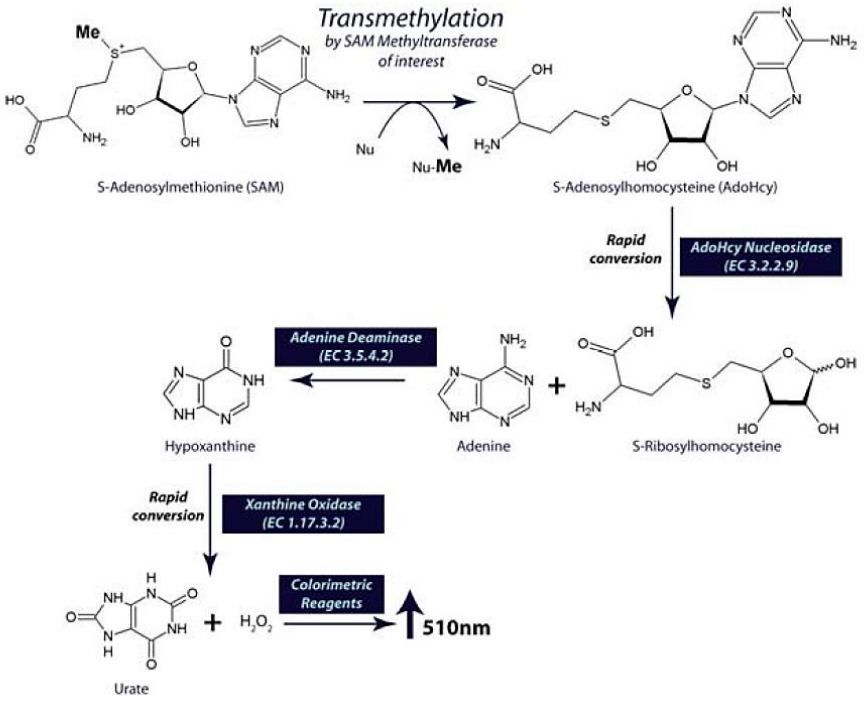

Figure 1. SAM510: SAM methyltransferase assay scheme

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 510 nm. 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.

Materials Provided

Description	Volume
SAM Methyltransferase Assay Buffer	20 mL
SAM Methyltransferase Assay Buffer Additive	0.2 mL
SAM Enzyme Mix	3 x 300 µL
SAM Colorimetric Mix	1
Positive Control: Adenosylhomocysteine (1 mM)	0.2 mL
S-Adenosylmethionine	3 vials
HCl Assay Reagent (20 mM)	1 mL

Materials Required (Not supplied)

Reagents

- Purified SAM Methyltransferase to be tested.
- Appropriate methyltransferase acceptor substrate.

Equipment

Plate reader suitable for half volume (115 µL) to measure between 500-520 nm absorbances.

Storage and Stability

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.

Protocol

Important Information

- The final volume of the assay is 115 µL 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

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 5 µL of your SAM methyltransferase samples to at least two wells of a 96-well plate. Use the SAM Methyltransferase Assay Buffer or 0.1 M Tris, pH 8.0 as a diluent. We recommend performing the reactions and controls in at least duplicate.

   For the background control
   Aliquot 5 µL SAM Methyltransferase Assay Buffer into each background control well. We recommend performing the reactions in duplicate.

   For the positive control
   Add 5 µL Positive Control and 10 µL SAM Methyltransferase Assay Buffer to each positive control well. We recommend performing the reactions in duplicate.

3. Add 10 µL the appropriate acceptor substrate to the sample and background control wells, using SAM Methyltransferase Assay Buffer or 0.1 M Tris, pH 8.0 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 10 µL.

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	3 mL	6 mL	9 mL
SAM Enzyme Mix	1 vial/300 µL	2 vials/600 µL	3 vials/900 µL
SAM Colorimetric Mix	200 µL	400 µL	600 µL
S-Adenosylmethionine	1 vial/100 µL	2 vials/200 µL	3 vials/300 µL

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

Data Analysis

1. Calculate the average absorbance of each sample.
2. Calculate the change in absorbance (ΔAbs) per minute, by:
   - 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.
   - Or calculate the change in absorbance between two points on the linear portion of the curve using the following equation:

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

3. Calculate the rate of ΔAbs/min for the background control wells and subtract this rate from the sample well rate.
4. Calculate Methyltransferase Activity: Use the following equation to calculate the Methyltransferase activity:

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

   The rate of the reaction is determined using the 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) extinction coefficient 15.0 mM^{-1*}. One unit of Methyltransferase will transfer 1.0 µmol 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 $mM^{-1}cm^{-1}$. This value has been adjusted for the pathlength of the solution in the well (0.577 cm).
5. If activators or inhibitors were assayed, determine the percent activation/inhibition for each sample as follows:

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

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

Assay Range

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

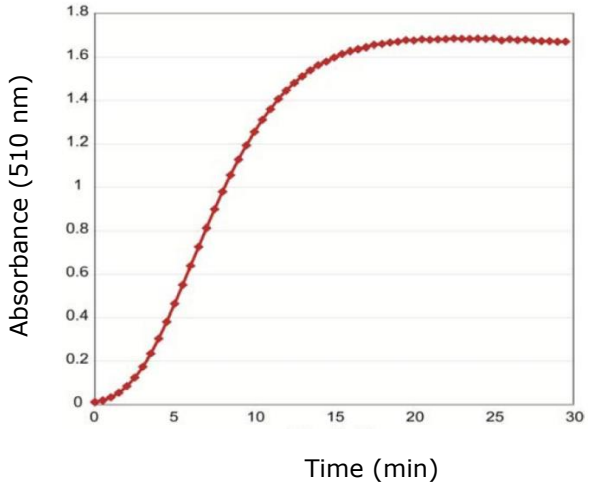

Figure 2. SAM510 Assay quantitatively assays the Positive Control: 50 µM Adenosylhomocysteine (AdoHcy).

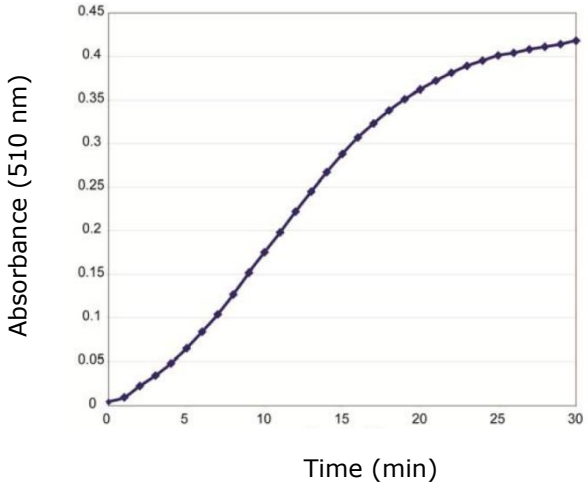

Figure 3. Human lysine specific histone Methyltransferase SET7/9 assayed with 20 µM TAF-10 as the acceptor substrate.

Troubleshooting

Problem	Cause	Solution
Erratic values; dispersion duplicates/triplicates	Poor pipetting technique	Use a repeating pipette and do not eject solution too vigorously
	Air bubbles in well	Gently tap plate to dislodge air bubbles
No absorbance detected above background control	Sample too dilute or acceptor substrate not added	Re-assay using a lower dilution and ensure acceptor substrate is added
	Acceptor substrate interferes with assay	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 0.1 M Tris, pH 8.0
No inhibition or activation was seen with added compounds	The compound concentration was too low	Increase compound concentration and re-assay
	Compound is not an inhibitor or activator	

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.

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