UV, Colorimetric & Fluorescence Continuous Enzyme Assays to Measure Activity of SAM Methyltransferases

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 (Cheng & Blumenthal).

The S-adenosylmethionine (SAM) dependent methyltransferases use SAM, the second most commonly used enzymatic cofactor after ATP. SAM, also known as AdoMet or SAMe, 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 (Schubert et al).

The SAM Methyltransferase Assays are continuous enzyme coupled assay that can continuously monitor SAM-dependent methyltransferases (Dorgan et al) without the use of radioactive labels or endpoint measurements.

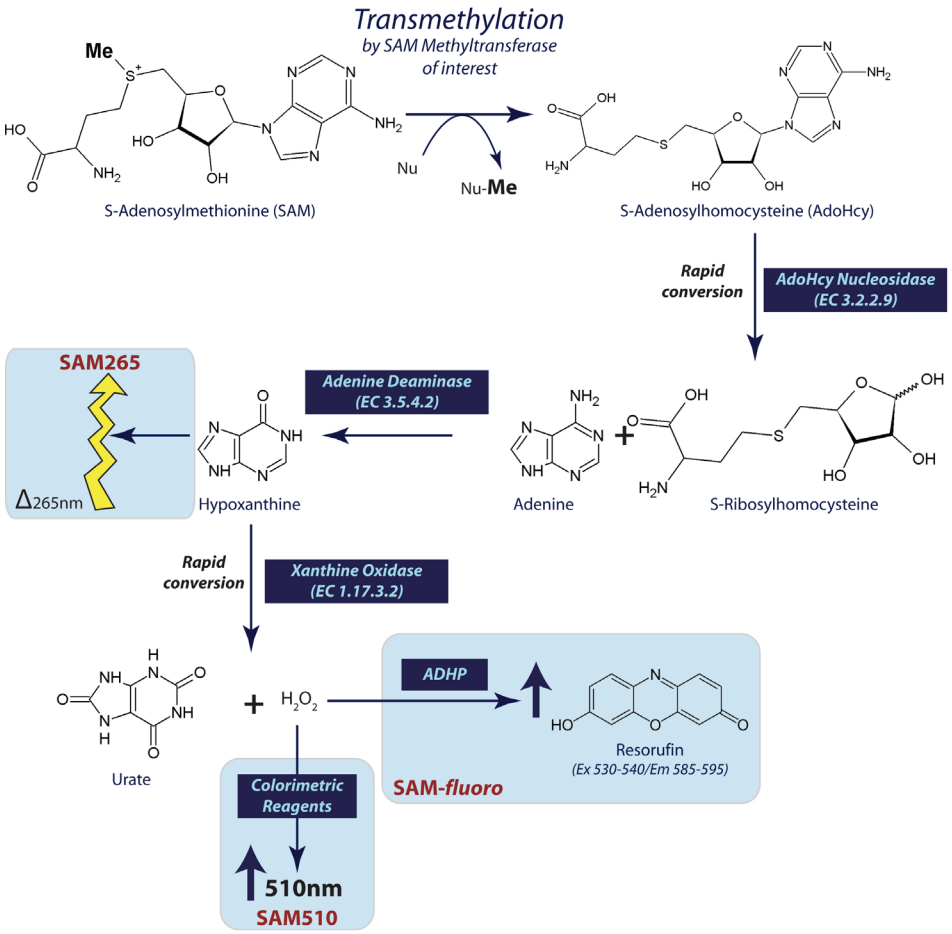
AIM

To demonstrate the non-radioactive continuous enzyme assays for S-adenosylmethionine dependent methyltransferases.

The methyltransferases transfer a methyl group from SAM to a specific molecule resulting in the production of S-adenosylhomocysteine (AdoHcy) (Figure 1).

The majority of methyltransferases assays utilize the SAM substrate labeled with either ^{14}C or ^{3}H, which in turn require subsequent time-consuming separation of the AdoHcy product from the substrate. An additional drawback with these assays is that the AdoHcy product can act as a potent feedback inhibitor to the methyltransferase, resulting in an increase in the errors experienced in determining the kinetic parameters.

Figure 1: SAM Methyltransferase Assays General Scheme



The key component of the SAM Methyltransferase Assay is AdoHcy Nucleosidase that rapidly catalyses the release of adenine from AdoHcy (or 5-methylthioadenosine (MTA)). The release of adenine blocks the feedback inhibition of AdoHcy on the methyltransferase. A second enzyme, adenine deaminase, is present to convert adenine to hypoxanthine.

The SAM265: SAM Methyltransferase Assay monitors the resulting absorbance decrease by monitoring at 265nm with UV spectrometry (Figure 1).

The SAM510: SAM Methyltransferase Assay and SAM-*fluoro:* SAM Methyltransferase Assay incorporate a third enzyme, xanthine oxidase, to convert hypoxanthine to urate and hydrogen peroxide (Figure 1). The resulting hydrogen peroxide is detected with colorimetric reagents or the fluorophore ADHP respectively.

METHOD

The general method is represented by the scheme shown in figure 1. The assays can be adapted to be used with any SAM dependent methyltransferase or an enzyme reaction that produces 5-adenosylhomocysteine or 5'-methylthioadenosine, due to the specificity of adenosylhomocysteine nucleosidase.

SAM265

0-3µM thiopurine S-methyltransferase was assayed with the SAM265 Assay using thiophenol as the methyl acceptor, according to the supplied protocol.

SAM510

Human lysine-specific histone methyltransferase SET7/9 was assayed with the SAM510 Assay using 20µM TAF-10 as the methyl acceptor, according to the supplied protocol.

SAM-fluoro

Human lysine-specific histone methyltransferase SET7/9 was assayed with the SAM-*fluoro* Assay using 20µM TAF-10 as the methyl acceptor, according to the supplied protocol.

RESULTS AND DISCUSSION

SAM265

The results of the assay were plotted on a graph as a change in absorption at 265nm versus time in minutes.

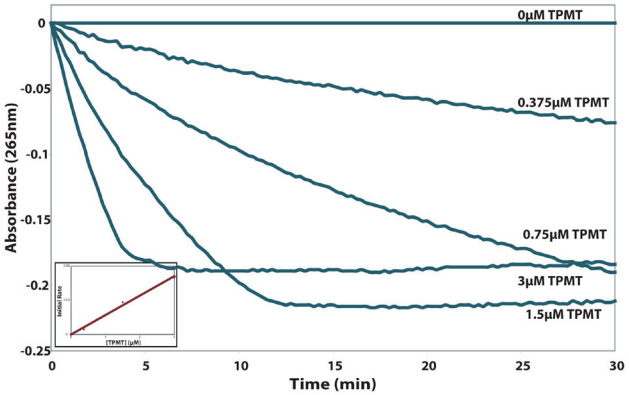


Figure 2: SAM265™ Assay quantitatively assays Adenosylhomocysteine (AdoHcy). *0-100µM AdoHcy was assayed with SAM265™. The inset graph shows that there is a linear correlation between absorbance change at 265nm and AdoHcy concentration.*

SAM510

The results of the assay were plotted on a graph as a change in absorption at 510nm versus time in minutes.

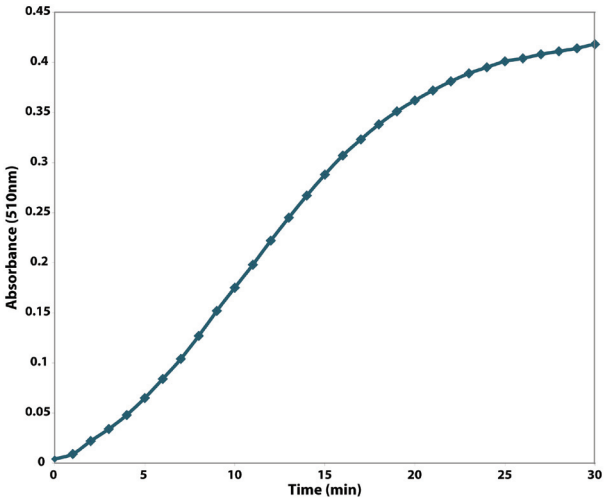


Figure 3: SAM510™ Assay quantitatively assays human lysine specific histone methyltransferase SET7/9(EC 2.1.1.43). *Human lysine specific histone methyltransferase SET7/9 was assayed with SAM510: SAM Methyltransferase Assay using 20µM TAF-10 as the methyl acceptor.*

SAM-fluoro

The results of the assay were plotted on a graph as a change in fluorescence versus time in minutes.

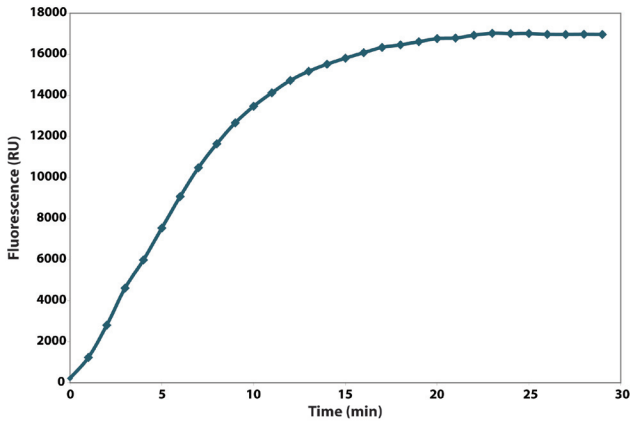


Figure 4: SAM-fluoro™ Assay quantitatively assays human lysine specific histone methyltransferase SET7/9(EC 2.1.1.43). *Human lysine specific histone methyltransferase SET7/9 was assayed with SAM-fluoro: SAM Methyltransferase Assay using 20µM TAF-10 as the methyl acceptor.*

The graphs demonstrate that these continuous assay can be used to characterize enzyme activity of SAM dependent methyltransferases.

SAM 265 assay is highly versatile for proteins, there are some potential limitations. The first is the assay is monitored at 265nm and therefore the presence of methyltransferase substrates that strongly absorb at 265nm, such as DNA, is problematic. The presence of such substrates would result in a narrow range of absorbance changes and analysis will be subject to the detection limit of the UV spectrophotometer. This assay has been successfully used to detect the change of 35µM AdoHcy to hypoxanthine in the presence of 4mM dNTPs (Schubert et al).

To combat this issue, the colorimetric and fluorometric SAM Methyltransferase Assay,are offered.

The SAM Methyltransferase Assays are designed for SAM methyltransferases but can be readily adapted to assay other enzymes that produce AdoHcy or methylthioadenosine products. Table 1 lists some of the enzymes that fall into this category.

EC No.	Official Name	Reaction Catalysed
1.16.1.8	[methionine synthase] reductase	[Methionine synthase]-cob(II) alamin + NADPH + S-adenosyl-L-methionine = [Methionine synthase]-methylcob(I)alamin + **AdoHcy** + NADP+
2.1.1.1-157*	S-Adenosylmethionine dependent methyltransferases	S-adenosyl-L-methionine + substrate = **AdoHcy** + methylated substrate
2.5.1.4	Adenosylmethionine cyclotransferase	S-adenosyl-L-methionine = **MTA** + 2-aminobutan-4-olide
2.5.1.16	Spermidine synthase	S-adenosyl-(5')-3-methylthio-1-propylamine + 1,4-diaminobutane = **MTA** + spermidine
2.5.1.22	Spermine synthase	S-adenosylmethioninamine + spermidine = **MTA** + spermine
2.5.1.23	Sym-norspermidine synthase	S-adenosylmethioninamine + propane-1,3-diamine = **MTA** + N-(3-aminopropyl)-1,3-diaminopropane
2.5.1.24	Discadenine synthase	S-adenosyl-L-methionine + N6-(delta2-isopentenyl)-adenine = **MTA** + discadenine
2.5.1.25	tRNA-uridine aminocarboxypropyltransferase	S-adenosyl-L-methionine + tRNAPhe = **MTA** + tRNA 3-(3-amino-3-carboxypropyl) uridine
2.5.1.38	Isonocardicin synthase	S-adenosyl-L-methionine + nocardicin E = **MTA** + isonocardicin A
2.3.1.161	Lovastatin nonaketide synthase	acetyl-CoA + malonyl-CoA + NADPH + H^+ + S-adenosyl-L-methionine = dihydrononacolin L + CoA + CO_2 + $NADP^+$ + **AdoHcy**
3.3.1.2	Adenosylmethionine hydrolase	S-adenosyl-L-methionine + H_2O = **MTA** + L-homoserine
4.4.1.14	1-aminocyclopropane-1-carboxylate synthase	(-)-S-adenosyl-L-methionine = **MTA** + 1-aminocyclopropane-1-carboxylate

Table 1: Enzymes that produce S-adenosylhomocysteine (AdoHcy) or methylthioadenosine (MTA). *The enzymes in the table produce the products for the SAM Methyltransferase Assay and therefore the assay may be suitable to assay the above enzymes. *Does not include 2.1.1.3, 5, 14, 19, 21, 23, 24, 30, 45, 54, 58, 63, 73, 74, 81, 86, 90, 134, 135, 138, 148. (Data compiled with aid of Brenda, The Comprehensive Enzyme Information System).*

ORDERING INFORMATION

VWR Cat. No.	Description	Size
95029-230	SAM265™: SAM Methyltransferase Assay	100 Assays
95029-232	SAM510™: SAM Methyltransferase Assay	100 Assays
89130-992	SAMfluoro: SAM Methyltransferase Assay	100 Assays

REFERENCES

1. Kumar, A. et al (2011) J. Biol. Chem. 286:19652
2. Aktas, M. and Narberhaus, F. (2009) J. Bacteriol. 191: 2033
3. Dorgan, K.M. et al. (2006) Anal. Biochem. 350:249
4. Schubert, H.L. et al. (2003) Trends Biochem. Sci 28: 329
5. Cheng, X. & Blumenthal, R.M. (1999) S-Adenosylmethionine Dependent Methyltransferases World Scientific, Singapore