Supporting Information

High-Throughput Assays for SAM-Dependent Methyltransferases: Advances, Challenges, and Future Perspectives

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Assay Principle	Detected Compound	Method	Enzyme Preparation	Detection Limit/Range	Comments	Reference
L-Homocysteine release from *S*-ribosylhomocysteine by lyase	L-homocysteine	Colorimetric Assay 412 nm	Purified Enzyme	4 µM SAH 5 to 200 µM SAH	Non-continuous assay	[1]
L-Homocysteine release from SAH by hydrolase	L-homocysteine	Colorimetric Assay 412 nm	Purified Enzyme	0.4 µM SAH 4 to 250 µM SAH	Non-continuous assay, 1h incubation with DTNB	[2]
Adenine release by SAH nucleosidase followed by deamination	Hypoxanthine	Spectrophotometric Assay 265 nm	Purified Enzyme	35 µM SAH below 250 µM	Susceptible to background interference at 265 nm	[3]
Release of ammonia coupled with NAD(P) oxidation	$NAD(P)^+$	Spectrophotometric Assay 340 nm	Purified Enzyme	170 nM SAH n.m.	Increased sensitivity by coupling with NAD(P)H oxidation, three additional enzymes necessary for the assay	[4]
Release of ammonia coupled with NAD(P) oxidation	$NAD(P)^+$	Spectrophotometric Assay 340 nm	Purified Enzyme	n.m. up to 100 µM SAH	Reducing complexity by replacing SAH nucleosidase and adenine deaminase with SAH deaminase	[5]
Oxidation of TMB by chloroperoxidase oxidized iodide (HOI)	Iodide	Colorimetric Assay 570 nm	Purified Enzyme Crude lysate	n.m. 5 to 400 µM Iodide	Insensitive to chloride, compatible with crude lysate, halide methyltransferases only	[6]
Peroxidase-mimicking Ag/Pt nanocluster	Methylated DNA	Colorimetric Assay 652 nm	Purified Enzyme	0.05 U/ml 0.5 to 10 U/ml	DNA methyltransferases only	[7]

Peroxidase-mimicking Ag/Pt nanoparticles	Methylated DNA	Colorimetric Assay 652 nm	Purified Enzyme	0.04 U/ml 0.2 to 2 U/ml	DNA methyltransferases only	[8]
Methylation of quinoline	1-methylquinoline (1-MQ)	Fluorescence Assay 330/405 nm	Purified Enzyme	40 nM 1-MQ 1 to 25 µM 1-MQ	Substrate specific fluorescence (1-MQ)	[9]
Oxidation of adenine by xanthine oxidase	H_2O_2	Fluorescence Assay 530/590 nm	Purified Enzyme	< 1 µM H_2O_2 up to 20 µM H_2O_2	Further experimentation necessary to assess feasibility for kinetic measurements (xanthine oxidase probably provides insufficient coupling activity)	[10]
SAH-sensing FRET riboswitch assay	SAH	Fluorescence Assay	Purified Enzyme	< 10 nM SAH n.m.	Suitable for accurate determination of IC_{50} values of inhibitors	[11]
SAH-sensing FRET RNA aptamer assay	SAH	Fluorescence Assay 485/600 nm	Purified Enzyme	< 18 nM SAH n.m.	Microscale thermophoresis-based assay requiring only 10 µL sample, 300-fold higher selectivity for SAH than SAM	[12]
Release of SH_2 from L-homocysteine by lyase	SH_2	Fluorescence Assay 365/450 nm	Purified Enzyme Crude lysate	< 1 µM SAH up to 60 µM SAH	Compatible with crude lysate	[13]
SAH hydrolase releasing L-homocysteine	L-homocysteine	Fluorescence Assay 340/450 nm	Purified Enzyme	n.m.	Miniaturized to 1536-well format	[14]
Ratiometric fluorescence signal of pyranine due to changes in H^+ concentration	H^+	Fluorescence Assay 405&450/510 nm	Purified Enzyme Crude lysate Pre-treated whole cell	~4–12 µM LOD up to 600 µM c(H^+)	Enzyme-uncoupled, continuous, high-throughput, susceptible to background proton fluctuations, high dependence on solution pH and p*K*a of compounds	[15]

Stepwise transformation of SAH to ATP via adenine and AMP	ATP	Bioluminescence Assay	Purified Enzyme	60 nM SAH 0.06 to 40 µM SAH	Suitable for endpoint and continuous measurement	[16]
Degradation of SAH to AMP under the consumption of ATP	ATP	Bioluminescence Assay	Purified Enzyme	n.m. 10 to 100 nM ATP	Assay volume of 10 µL, decreasing signal corresponds to higher MT activity	[17]
Two stage stepwise conversion of SAH to ADP, which is then phosphorylated to ATP	ATP	Bioluminescence Assay	Purified Enzyme	20 to 30 nM SAH	Non-continuous assay, one-step assay reaction using both reagents is possible	[18]
Transformation of SAH to ATP via MTaseGlo or hydrolysis of SAH to L-homocysteine	ATP and L-homocysteine	Bioluminescence Assay	Purified Enzyme	n.m.	Miniaturized to 1536-well format	[14]
In vivo conversion of SAH to L-homocysteine triggering GFPuV	GFPuV	Biosensor	Whole cell application	< 1 µM up to 100 µM L-homocysteine	High-throughput *in vivo* screening	[19]
Detection of SAH via mass spectrometry	SAH	LC-MS	Purified Enzyme Crude lysate	50 nM SAH 0.05 to 1.6 µM SAH	No reaction workup besides quenching with formic acid and centrifugation, not high-throughput	[20]

n.m. - not mentioned

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