Universal Homogeneous Bioluminescent Assay to Monitor the Activity of Various Classes of Methyltransferases in vitro

Michael Curtin, Kevin Hsiao[1], Said A. Goueli[1,2]

[1]Research and Development, Promega Corp. Madison, WI 53711, and [2]University of Wisconsin School of Medicine and Public Health, Madison, WI 53706

Abstract #130

1. Introduction

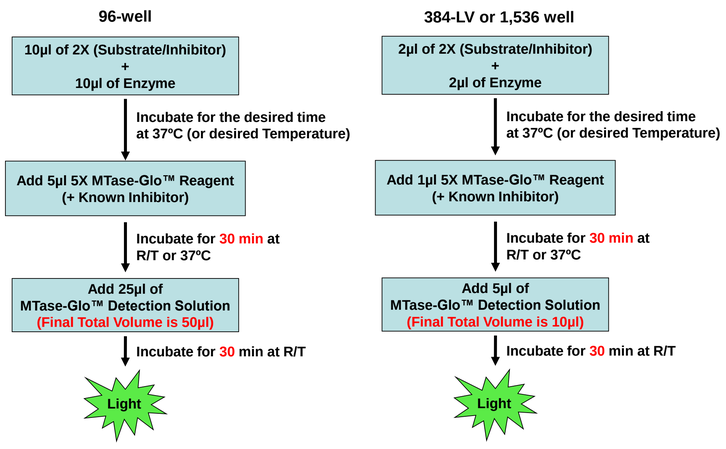
Post transcriptional modifications of proteins and nucleic acids are well-recognized as playing a major role in many cellular processes. Recent biochemical and biological data suggest that the enzymes involved in such modifications, including phosphorylation, acetylation, methylation, etc, play pathogenic roles in cancer, inflammation, and neurodegenerative diseases. Thus, pharmacological modulation of these enzymes by small molecules will be beneficial in developing novel therapeutics for multiple unmet medical needs. Of these, methyltransferases are known to alter the epigenome by altering the methylation status of nucleic acids or proteins resulting in changes in cellular functions. In order to screen for modulators of these enzymes for the development of next generation of drugs, robust screening assays are urgently needed. We have developed a novel assay that monitors the activities of methyltransferases and their modulation by small molecules.

The assay is universal since it is based on quantitation of S-adenosylhomocysteine (SAH), a product of most methylation reactions, thus can be used with of a broad range of methyltransferases such as DNA, protein, RNA, and small molecules methyltransferases. The bioluminescent assay is quite sensitive and is formatted for HTS applications. In addition, the assay has been validated for all classes of protein methyltransferases (Lysine and Arginine), and with different types of substrates (small peptides, large proteins, or even nucleosomes). A strong feature of this assay is its utility with a broad range of substrates with no limitations their concentration or composition (short vs. long peptides), thus enabling the generation of kinetic data and determining the mechanism of action of various modulators of methyltransferases of interest.

2. Universal Methyltransferase MTase-Glo™ Assay Flexibility

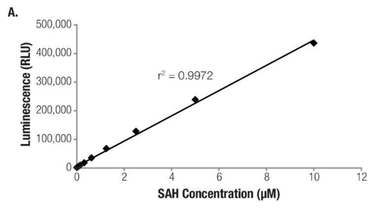
MTase-Glo™ Methyltransferase Assay Reaction

MTase-Glo™ Methyltransferase Assay Protocol

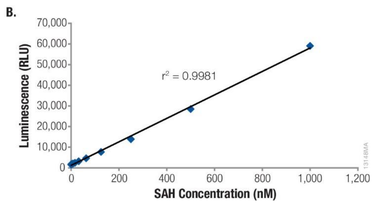


3. Sensitivity of MTase-Glo™ Assay

A. Low Range Up to 1µM SAH

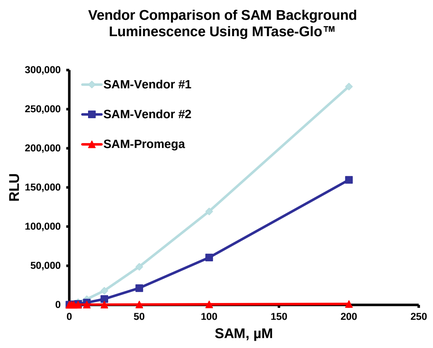


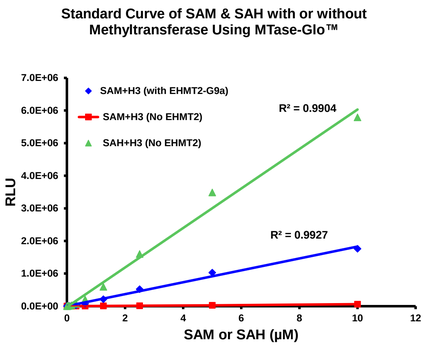
B. High Range Up to 10µM SAH



Representative SAH standard curves for the MTase-Glo™ Assay. Purified SAH at the indicated concentrations was dispensed into a low-volume 384-well plate. The MTase-Glo™ Assay was performed as described in Section 4.A of Promega Technical Manual TM#453. Luminescence was measured using a plate-reading luminometer.

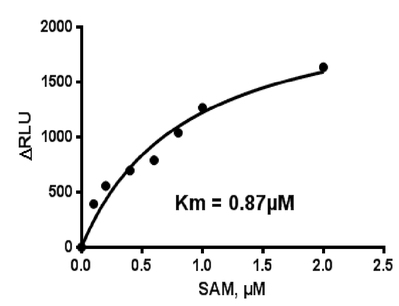
4. Quality of SAM Substrate is Critical for the Performance of Methyltransferase Assay



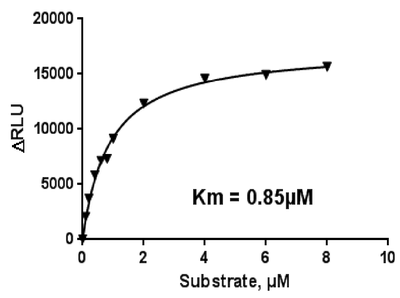


5. Kinetic Study: Titration of Substrates Using EHMT2-G9a And MTase-Glo™

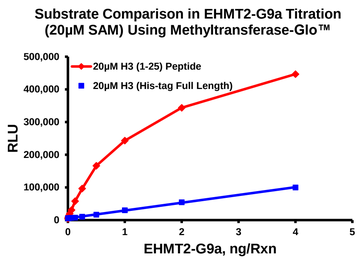
A. SAM with EHMT2-G9a

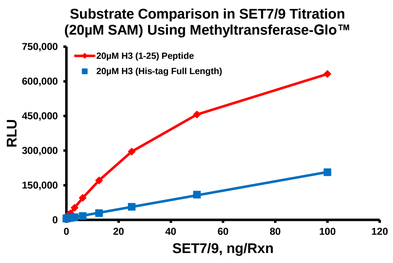


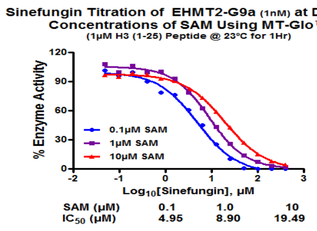
B. H3 (1-25) Peptide Substrate with EHMT2-G9a

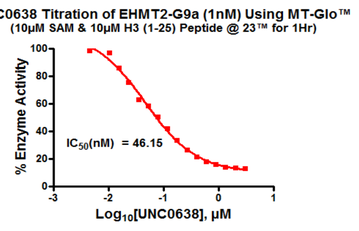


6. Methyltransferase Activity and Inhibitor Titration of EHMT2-G9a & SET7/9

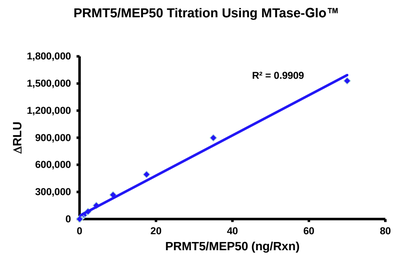


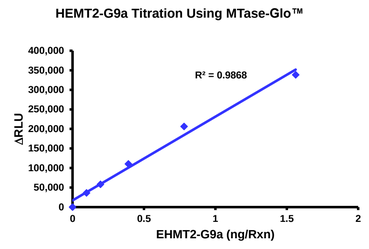


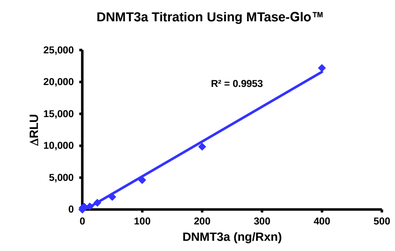


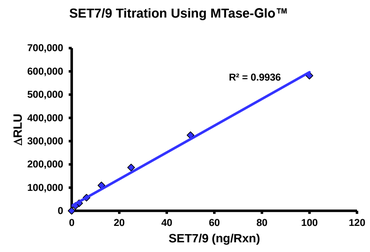


7. Methyltransferase Activity by Different Classes of Methyltransferase



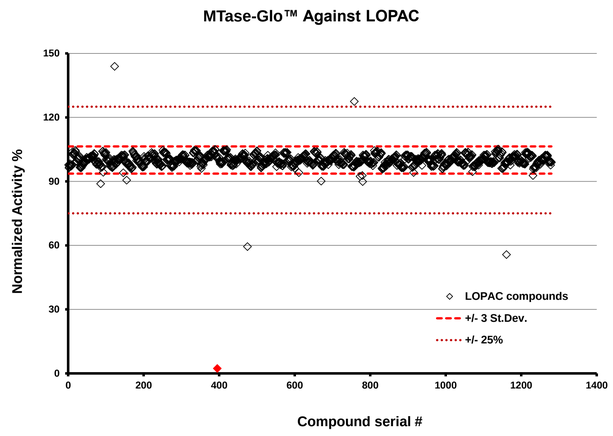






Titration of methyltransferases using the MTase-Glo™ Assay. PRMT5/MEP50 (Panel A), EHMT2-G9a (Panel B), DNMT3a (Panel C) or SET7/9 (Panel D) were assayed at the indicated concentration in low-volume 384-well plate. The MTase-Glo™ Assay protocol was performed as described in Section 4.A of Promega Technical Manual TM#453. RMT5/MEP50, EHMT2-G9a, and SET7/9 were obtained from BPS Bioscience, Inc. (San Diego, CA). DNMT3a was purchased from Reaction Biology Corp (Malvern, PA).

8. LOPAC Screening Using MTase-Glo™ Assay



LOPAC Screening Using MTase-Glo™ Methyltransferase Assay. MTase-Glo was used to screen the LOPAC Library (Library of Pharmacologically Active Compounds). The vast majority of compounds had no effect on the activity of the MTase-Glo Assay- the three compounds that did affect the activity of the assay more than 40% were all known luciferase inhibitors.

9. Conclusions

MTase-Glo™ Methyltransferase Assay

Highly Sensitive:

- Sensitive: Detects Low SAH (< 40nM)
- Broad range of concentrations of SAM can be used in MTase-Glo™
- High S/B: >50 using 384LV-well format

Robust:

- Z' > 0.8 (at 1µM of SAH using 384LV-well format)

Universal Assay:

- Any Substrate: Histone derived peptides, Histone 3, Histone 4, DNA, or Nucleosomes as substrates
- Any Format: 96-, 384-, 384LV, and 1536-well plates
- Minimal interference from fluorescent compounds
- Volume flexibility (as 4:1:5 ratio)
- Fast performance: ~60min after completion of MTase reaction

Corresponding author: michael.curtin@promega.com