

Overview

Methylation is a ubiquitous covalent modification used to control the function of diverse biomolecules including hormones, neurotransmitters, xenobiotics, proteins, nucleic acids and lipids. Histone methyltransferases (HMTs) are currently of high interest as drug targets because of their role in epigenetic regulation, however most HMT assay methods are either not amenable to an HTS environment or are applicable to a limited number of enzymes. We developed a generic methyltransferase assay method using fluorescent immunodetection of AMP, which is formed from the MT reaction product S-adenosylhomocysteine in a dual enzyme coupling step. The assay format shows >100 mP signal with Z' > 0.5 at initial rate conditions for 1 μM to 50 μM SAM. The suitability for HTS is demonstrated using 384-well plates with >16 hour deck (prior to plate addition) and signal (stability after plate addition) stability at room temperature. The activity of three HMTs (G9a, Set7/Set9, SUV39H1) were followed with using histone H3 peptides while G9a activity was assessed using both peptide and full length histone H3. In addition, inhibitor potencies were determined with G9a HMT. By combining a novel enzymatic coupling step with the well characterized Transcreener® AMP²/GMP² Assay, we have developed a robust HTS assay for HMTs which should be broadly applicable to other types of methyltransferases as well.

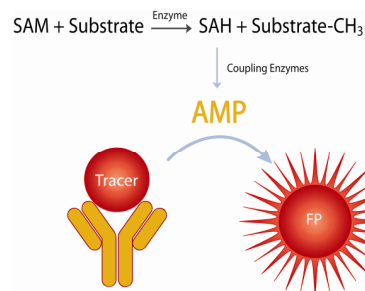


Figure 1. The Transcreener® EPIGEN Methyltransferase Assay Principle. SAH produced by the target methyltransferase is converted to AMP by coupling enzymes which allows homogenous fluorescent detection using the Transcreener® AMP²/GMP² Assay.

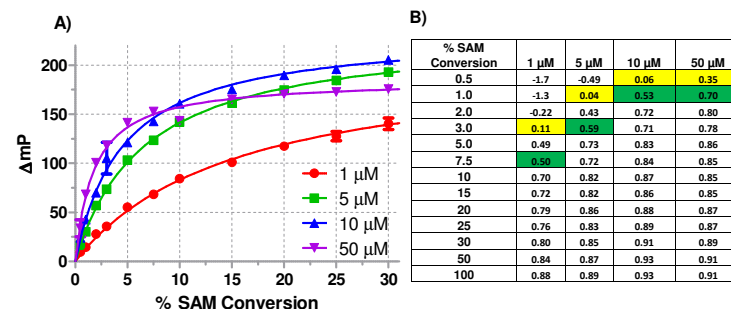


Figure 2. Initial Velocity Detection from 1 μM to 50 μM SAM. A) Standard curves mimic MT reactions (formation of SAH from SAM) show large assay window (>100 mP signal) at less than 20% SAM conversion over the full range of initial SAM concentrations tested. B) Table with Z' values at percent SAM conversion showing the lower limit of detection (yellow) and initial values that are ≥0.5 (green).

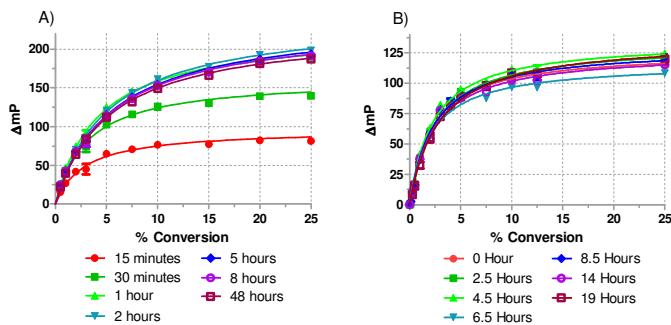


Figure 3. Signal and Reagent Stability for >16 hours. SAM/SAH standard curves (10 μM initial SAM) were used to examine A) equilibration and stability of signal after addition to coupling enzyme reactions and B) stability of pre-combined detection reagents prior to addition to the plate. Both studies were performed at room temperature.

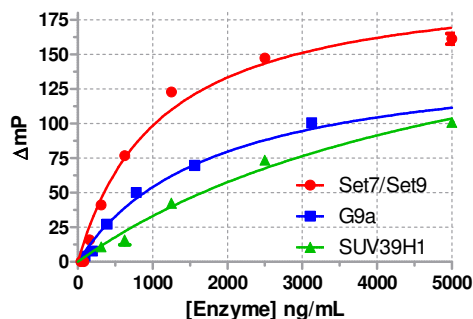


Figure 4. Universal HMT Detection. Enzyme titrations demonstrate dose responses for three HMTs, Set7/Set9, G9a, and SUV39H1 using Histone H3 peptide (1-25) at the SAM K_m (6 μM, 2 μM, and 12 μM, respectively). HMT reactions were run for 60 minutes followed by quench and detection reagents including the SAM-SA-H coupling enzyme mixture and the Transcreener® AMP²/GMP² monoclonal antibody and tracer.

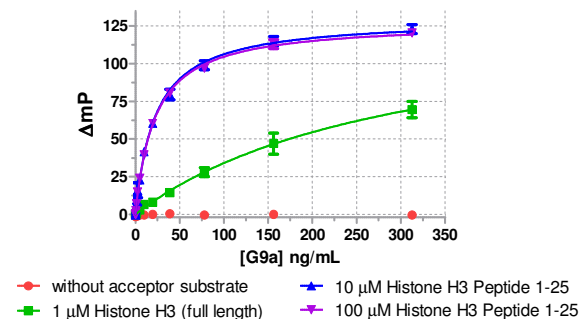


Figure 5. Accommodation of Different Acceptor Substrates. G9a histone methyltransferase reactions were performed with native protein (1 μM) or peptide substrates (10 μM or 100 μM) at 1 μM SAM. Reactions were processed as described in Figure 4.

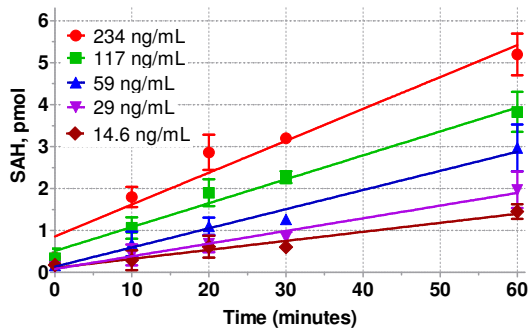


Figure 6. G9a Histone Methyltransferase Initial Rates. Initial velocity conditions were determined using saturating histone H3 peptide (1-25) of 50 μM with [SAM] at the K_m = 2 μM. Quantity of SAH was determined using a 2 μM SAM/SAH standard curve as shown in Figure 2.

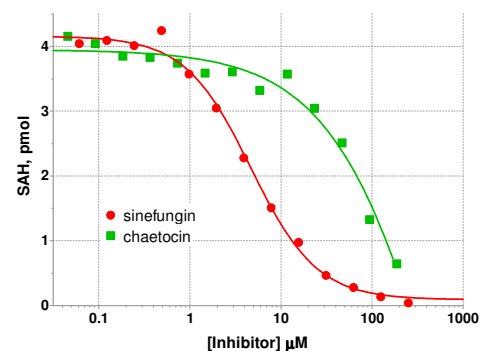


Figure 7. Pharmacology. Dose response curves were generated for G9a methyltransferase sensitivity with known inhibitors (chaetocin and sinefungin). The IC₅₀ values for sinefungin and chaetocin were 4.7 and 770 μM, respectively. The G9a enzyme reactions progressed to 25% SAM conversion with a Z' > 0.5.

Conclusions

- The Transcreener® EPIGEN Methyltransferase Assay combines coupling enzymes with the well characterized Transcreener® AMP²/GMP² FP reagents to enable universal homogenous methyltransferase enzyme detection.
- Excellent Z' values and >100 mP signal are achieved using [SAM] from 1 μM to 50 μM under initial velocity conditions.
- Greater than 16 hour deck and signal stability ensures compatibility with automated HTS infrastructure.
- The universal nature of the assay is demonstrated using three HMTs with either peptide or full-length protein substrates in a single assay.
- Inhibitor potency measurements were demonstrated using G9a HMT and two known HMT inhibitors, sinefungin and chaetocin.

Acknowledgements

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