Validation of Transcreener® EPIGEN Assays for Screening Chromatin Modifying Enzymes: Methylation and Acetylation

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Overview

Histone methyltransferases (HMTs), DNA methyltransferases (DNMTs) and Histone acetyltransferases (HATs) are currently of high interest as drug targets because of their role in epigenetic regulation, however most HMT/HAT assay methods are either not amenable to an HTS environment or are applicable to a limited number of enzymes. Transcreener® EPIGEN Assays are universal assay methods for methyltransferases and acetyltransferases based on detection of S-adenosylhomocysteine (SAH) and Coenzyme A (CoA) respectively. Coupling enzymes are used to convert the SAH or CoA to AMP which is detected using the Transcreener AMP² Assay, a competitive fluorescence polarization immunoassay that has been extensively validated in pharma HTS applications. In this poster we show the sensitivity and signal stability of the MT and AT assay. The lower limit of detection (LLD) for SAH in methyltransferase reactions utilizing 50 µM SAM is 0.25 µM SAH, and reactions containing 0.1 µM SAM has a LLD of 0.01 µM SAH. The assay signal is stable for almost 48 hours making the method very flexible for automated HTS protocols. We demonstrate the use of the EPIGEN MT and AT assays with diverse enzymes like-G9a, SET7/9, PRMTs, DNMT1, pCAF and GCN5. By combining a novel enzymatic coupling step with the well characterized Transcreener® AMP²/GMP² assay, we have developed and validated a robust HTS assay that is sensitive and generic and can be used for a variety of HMTs, DNMTs and ATs.

The Transcreener® EPIGEN Assays

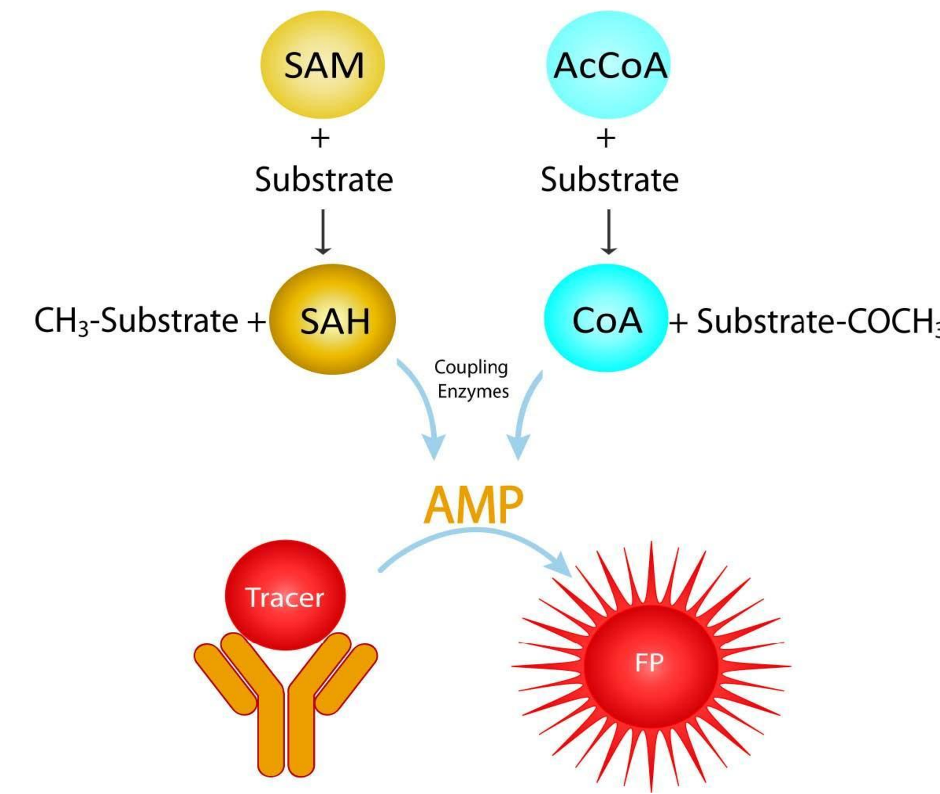

Figure 1. Assay Principle. The Transcreener® EPIGEN Assays combine the extensively validated Transcreener® AMP²/GMP² Assay with coupling enzymes that convert the SAH and CoA produced in Methyltransferase and Acetyltransferase reactions to AMP.

Standard Curves for EPIGEN Assays

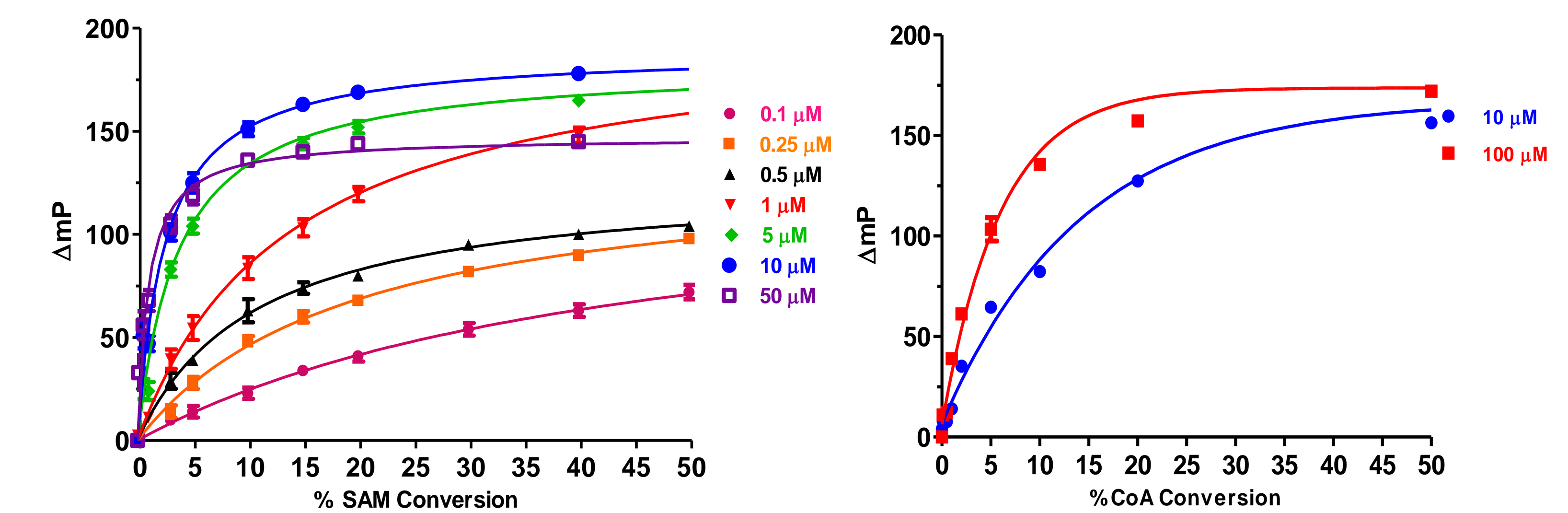

Figure 2. Excellent assay window for initial velocity detection upto 50 µM SAM and CoA. **(A)** Standard curves mimicking MT reactions (formation of SAH from SAM) show >100 mP signal at less than 20% conversion over the full range of initial SAM concentrations tested. **(B)** Standard curves mimicking AT reactions (formation of CoA from ACoA) show >100 mP signal at less than 20% conversion over the full range of initial ACoA tested.

Plate Stability

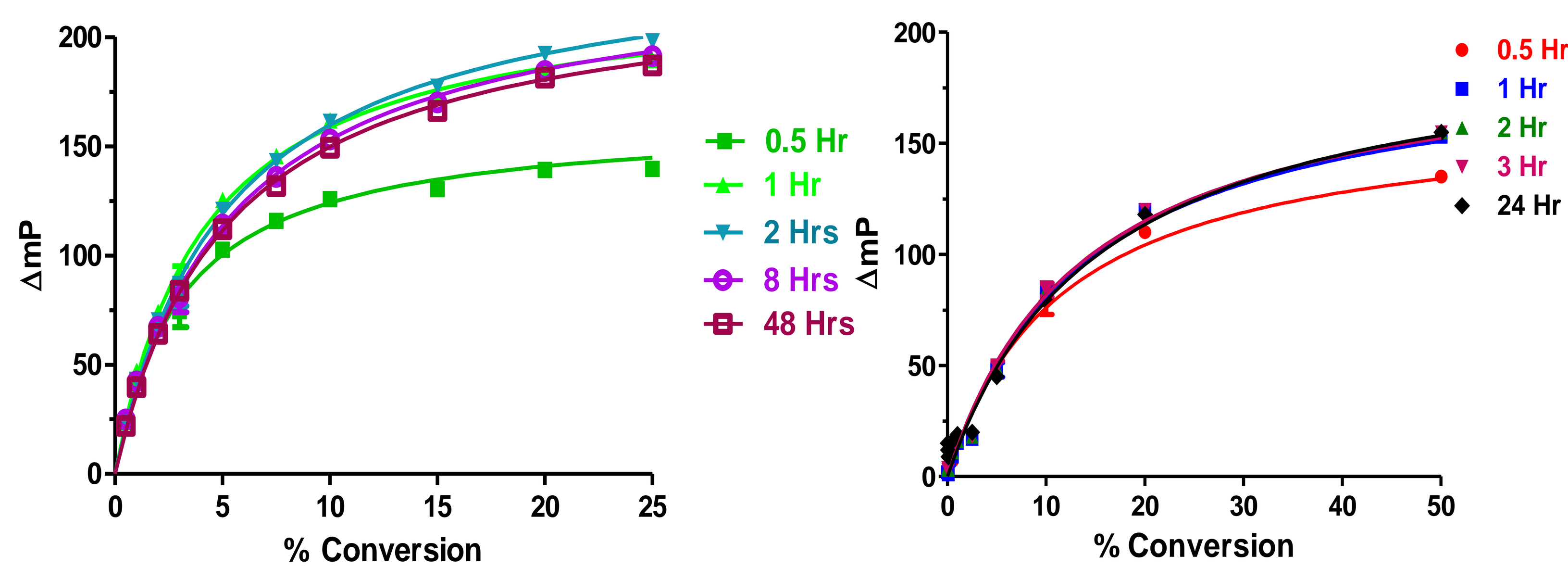

Figure 3. Reagent and Signal Stability of more than 12 hours. **(A)** SAM/SAH standard curves (10 µM initial SAM) were used to examine equilibration and stability of signal after addition to enzyme reactions. **(B)** ACoA/CoA standard curves (100 µM initial ACoA) were used to examine equilibration and stability of signal after addition to enzyme reactions.

Methyltransferase Titrations

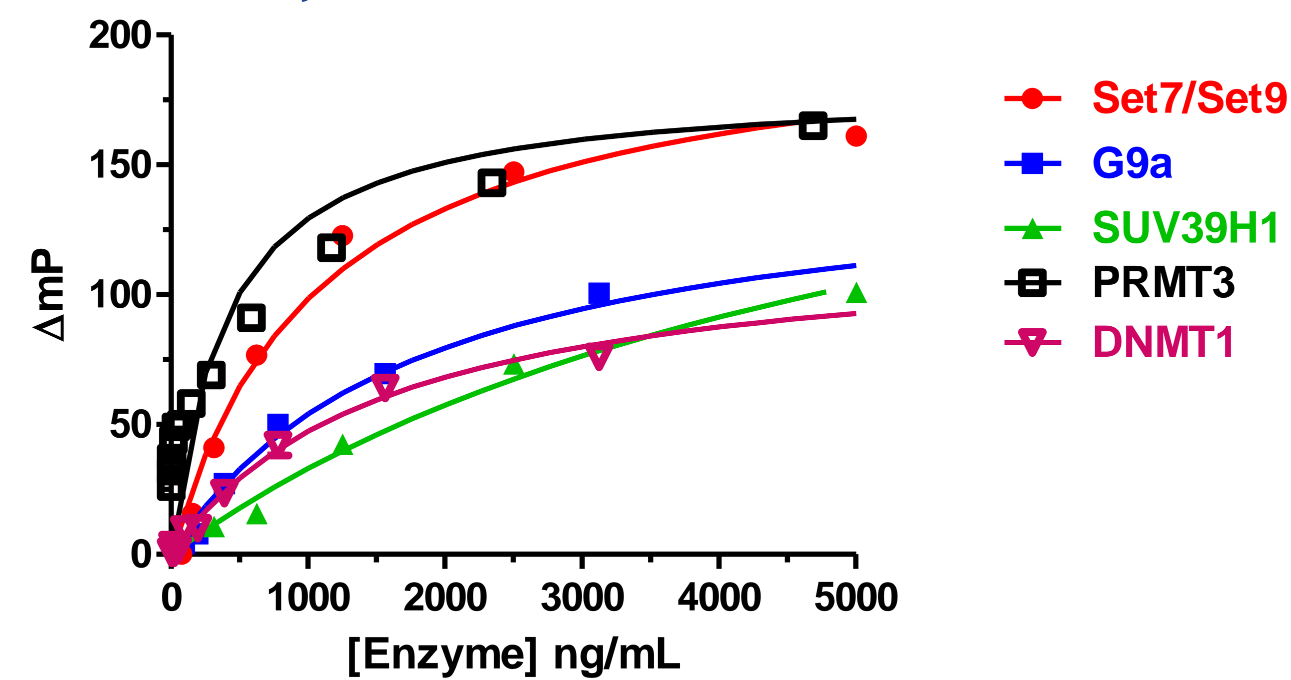

Figure 4. Universal MT Detection. Enzyme titrations demonstrate dose responses for HMTS-Set7/9, G9a, Suv39H1, PRMT3 and DNMT1 using Histone (For HMTs) and Poly d(I-C) for DNMT1 at the SAM K_m (6 µM, 2 µM, 12 µM, 3 µM and 2 µM respectively). MT reactions were run for 60 min followed by addition of detection/quench reagents including the SAM-SAH coupling enzyme mix and Transcreener® AMP²/GMP² Antibody and tracer.

Substrate Specificity

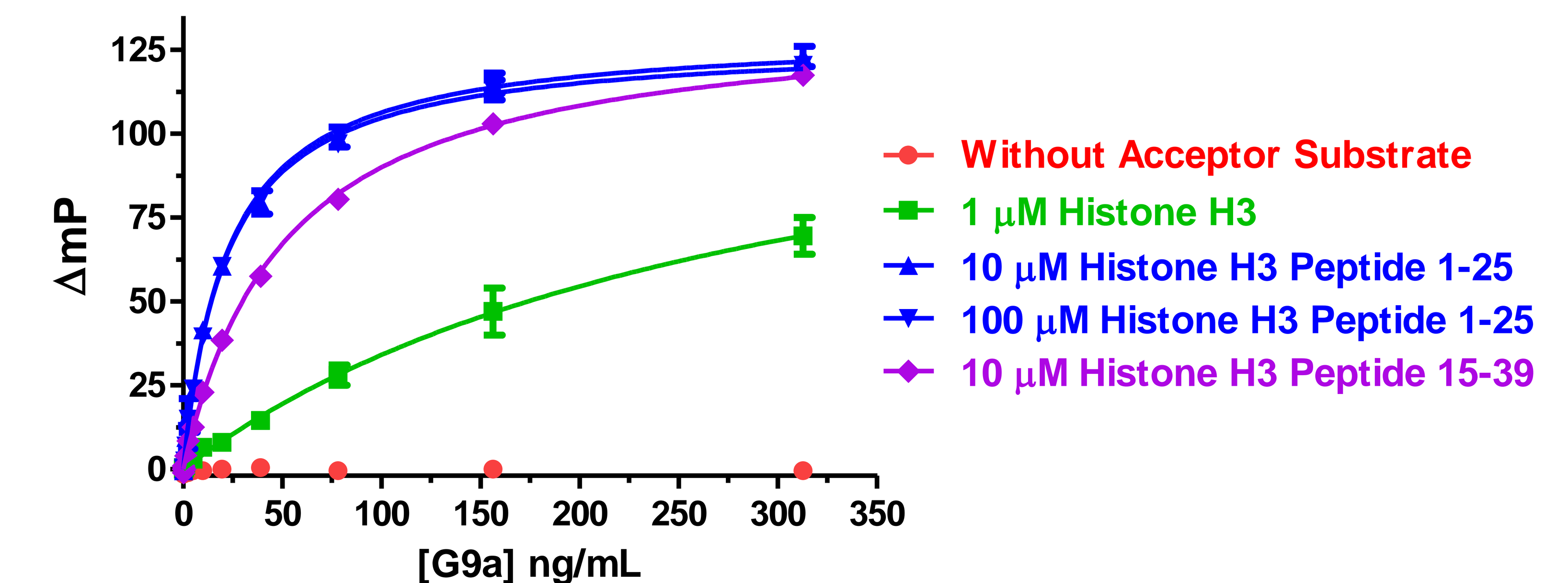

Figure 5. Accommodation of different acceptor substrates. G9a histone methyltransferase reactions were run with native protein (1 µM) or peptide substrates (10 µM or 100 µM) at 1 µM SAM. Reactions were processed as described in Figure 4.

Histone Acetyltransferases Titration

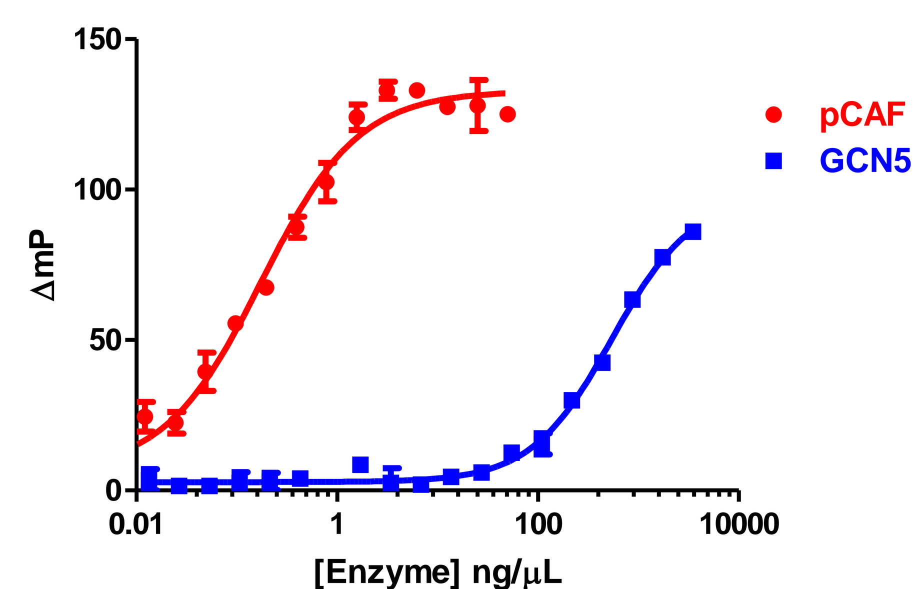

Figure 6. Universal HAT Detection. Enzyme titrations demonstrate dose responses for two HATs, pCAF and GCN5 using Histone H3 peptide (1-25) and 50 µM Acetyl CoA. HAT reactions were run for 60 min at 30°C followed by addition of detection/quench reagents and Transcreener® AMP²/GMP² Antibody and tracer.

Dose Response Curves

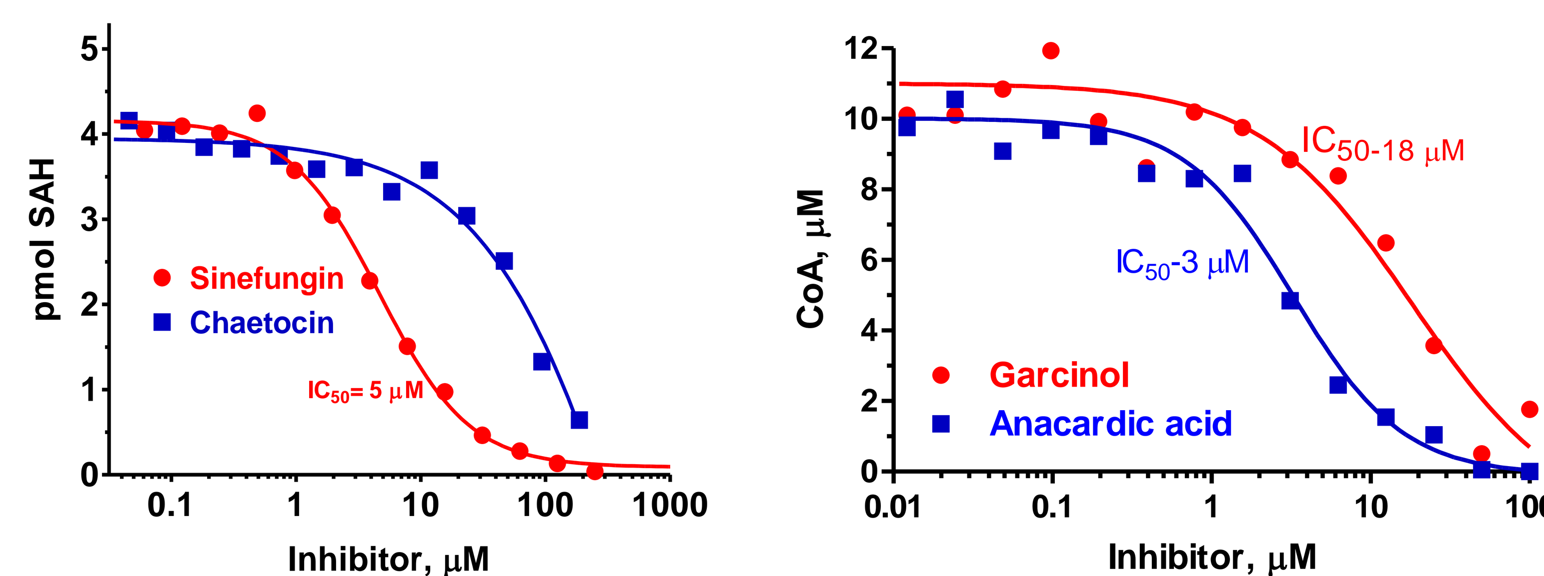

Figure 7. Correct pharmacology. **(A)** G9a methyltransferase sensitivity for known inhibitors (chaetocin and sinefungin) is shown for dose dependency curves. The IC_{50} value of sinefungin was determined to be 5 µM. **(B)** pCAF acetyltransferase sensitivity for inhibitors Garcinol and Anacardic acid is shown for dose dependency curves. The IC_{50} of Garcinol and Anacardic Acid were determined to be 18 µM and 3 µM respectively.

Conclusions

- The Transcreener® EPIGEN Assay is HTS compatible using Transcreener® AMP/GMP FP reagents used to follow the progress of enzymes that produce SAH or CoA.
- Excellent Z' values and large signal are shown for low percent conversion throughout the entire assay range from 0.1 µM to 50 µM SAM for methyltransferase assay and 10 µM to 100 µM CoA.
- The EPIGEN assays has greater than 19 hour deck and signal stability.
- The universal nature of the Transcreener® EPIGEN Assay is demonstrated allowing assessment of any methyl/acetyl transferase and any substrate using a single assay.
- Inhibitor pharmacology demonstrated using G9a histone methyltransferase and pCAF histone acetyltransferase.

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