Application Note

Detection of HMT PRMT1 with the Transcreener® EPIGEN Methyltransferase Assay

This Application note will serve as a guide for using the Transcreener® EPIGEN Methyltranferase assay to detect the initial velocity enzyme activity of HMT PRMT1 with an assay window suitable for inhibitor screening and dose response measurements. It should be as an adjunct to the EPIGEN Methyltransferase Assay

Histone Methyltransferase PRMT1

PRMT1 catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to an w-nitrogen of the guanidino function of protein L-arginine residues (w-monomethylation), and the second methyl group to the same nitrogen, yielding asymmetric dimethylarginine (aDMA)[2]. Human recombinant PRMT1 (residues 2-371 N-terminus); Genbank Accession # NM_001536) expressed as an N-terminal GST-tag protein in E.coli was obtained from Reaction Biology Corp (Malvern,PA).

Transcreener® EPIGEN Methyltransferase Assay

The Transcreener® EPIGEN Methyltransferase Assay is a universal biochemical HTS assay for enzymes that produce S-adenosylhomocysteine (SAH), including all enzymes within the histone (HMTs) and DNA (DNMTs) methyltransferase families. It combines the extensively validated Transcreener AMP²/GMP² Assay, which relies on fluorescent immunodetection of AMP, with coupling enzymes that convert SAH to AMP. Enzyme activity is signaled by a decrease in fluorescence polarization as the bound tracer is displaced from the AMP²/GMP² Antibody. The assay uses a simple mix-and-read format with two liquid addition steps. Methyltransferase (MT) enzyme reactions are first quenched with Stop Buffer and then the SAH Detection Mixture containing coupling enzymes, AMP²/GMP² antibody, and tracer is added. The assay provides excellent signal at low substrate conversion, with an assay window greater than 100 millipolarization units (mP) and Z' ≥ 0.7 under normal reaction conditions.

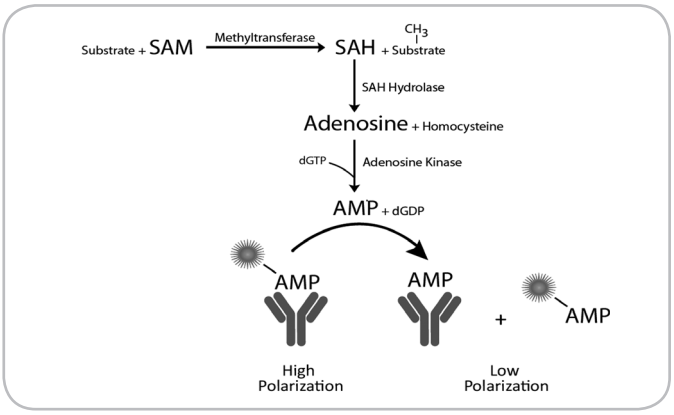

Figure 1. Transcreener Epigen methyltransferase assay principle: SAH produced in a methyltransferase reaction is converted to AMP in two sequential enzymatic steps. AMP is detected using a competitive fluorescence polarization immunoassay.

Summary of protocol

Step 1) Determine the optimal AMP²/GMP² antibody concentration for the PRMT1 enzyme reaction.

Step 2) Perform a PRMT1 titration to determine the amount required to produce a good assay window.

Step 3) Run dose response curves.

Materials and Methods

Instrument: Microplate Reader capable of measuring Fluorescence Polarization.

Microplates: Corning® 384 Well Low Volume Black Round Bottom PS NBS™ Microplate (Product #3676).

Note: Non binding or Low binding black plates are necessary for a good assay window.

Reagent	Catalog #
Transcreener® EPIGEN MT Assay	3017-1K
PRMT1 (Reaction Biology)	HMT-11-119
H4 peptide (1-20) (Anaspec)	62498

HMT Enzyme Buffer: 50 mM Tris-HCl (pH 8.5), 4 mM DTT, 0.1 M NaCl, 0.01% Triton X-100 and 5 mM $MgCl_2$.

Step 1) Determine the optimal AMP²/GMP² antibody concentration for the PRMT1 enzyme reaction.

Note:The optimal antibody concentration is primarily dependent on the SAM (2 µM) and the peptide (10 µM) concentration and to a lesser degree on other enzyme specific components such as metals and salts.

1) Titrate the AMP²/GMP² Antibody using a two fold dilution in a volume of 10 µL in the HMT Enzyme Buffer containing 2 µM SAM and 10 µM H4 peptide.

2) Add 10 µL of Detection Mix comprising AMP²/GMP² Tracer, Cofactor, Detection buffer, Coupling enzyme 1 and Coupling enzyme 2. The final concentrations of the components in 20 µL reactions were 4 nM tracer, 0.125X cofactor, 0.125X detection buffer, 2 µg/mL coupling enzyme 1 and 1 µg/mL coupling enzyme 2.

3) Mix the plate on a plate shaker, cover with a plate seal, and incubate at RT for an hour.

4) The plates were read at EXC 630 nm and EMS 670 nm to measure fluorescence polarization.

5) Plot polarization vs. log [Antibody] and determine the concentration that produces 85% of the maximal polarization change - the EC85; 5 µg/mL in this case. In general for Transcreener assays, using the EC85 antibody concentration will allow robust detection of enzyme initial velocity (less than 20% conversion of susbtrate to product).

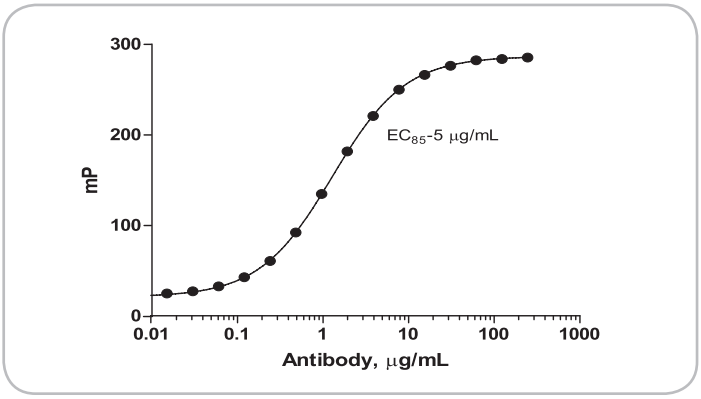

Figure 2. AMP²/GMP² antibody titration in the presence of 2 µM SAM and 10 µM H4 (1-20) peptide. An optimal concentration of 5 µg/mL was determined based on this titration.

Step 2) Perform a PRMT1 titration to determine the optimal enzyme concentration to use.

1) The final reaction volume is 15 µL; reactions are initiated by adding 7.5 µL of substrate to 7.5 µL of titrated enzyme. Titrate PRMT1 using two-fold dilutions by adding 7.5 µL aliquots to wells containing 7.5 µL enzyme buffer starting at 70 ng/µL and ending at 0 ng/µL (the maximum enzyme concentration will be 35 ng/µL after addition of substrate. We suggest running duplicates, with separate controls lacking the H4 peptide and SAM.

2) Initiate the reaction by adding 7.5 µL of substrate - Mix for final concentrations of 2 µM SAM and 10 µM H4 (1-20) peptide in the 15 µL reactions per the instructions in the Transcreener EPIGEN MT Assay Technical Manual. Prepare other controls by adding just SAM or H4 peptide to the titrated enzyme reactions.

3) Incubate the enzyme reaction for two hours at 30°C.

4) Meanwhile prepare a 2 µM SAM/SAH standard curve with 10 µM H4 peptide in the buffer at different percent conversions. Add 15 µL of the standards to the same plate and let them incubate at 30°C along with the enzyme reaction.

Note:For detailed instr.uctions on how to run a standard curve please refer to the EPIGEN Methyltransferase technical manual.

5) At the end of two hours, add 2.5 µL of Stop Buffer A to all the wells followed by 2.5 µL of detection mix. The detection mix comprises of AMP²/GMP² tracer, AMP²/GMP² antibody, cofactor, detection buffer, coupling enzyme 1 and coupling enzyme 2. The final concentrations of the components in 20 µL reactions were 4 nM tracer, 5 µg/mL of AMP² antibody, 0.125X cofactor, 0.125X detection buffer, 2 µg/mL coupling enzyme 1 and 1 µg/mL coupling enzyme 2.

6) Mix the plate well and after 90 min of incubation read the plate in an instrument that measures fluorescent polarization at 633 nm EXC and 670 nm EMS.

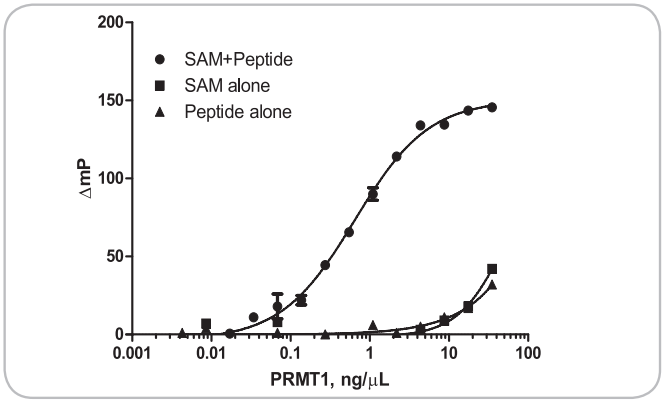

Figure 3. PRMT1 enzyme titration in the presence of 2 µM SAM and 10 µM H4 (1-20) peptide . A concentration (EC_{80}) of 2.9 ng/µL was determined optimal based on this titration.

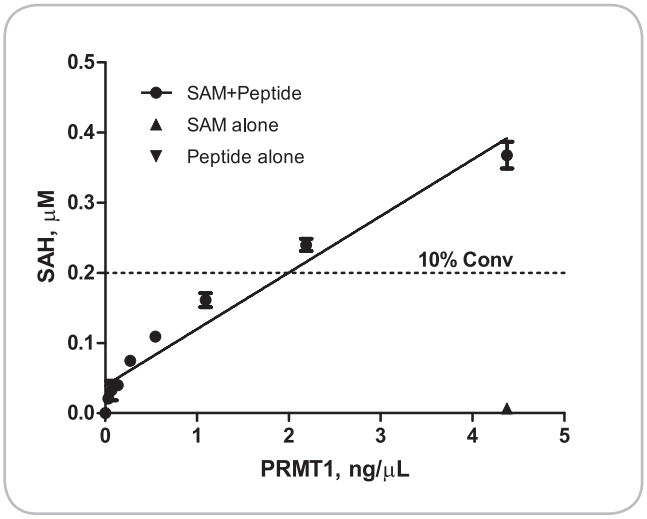

Figure 4. Linear relationship between PRMT1 concentration and SAH formation. The polarization values were converted into SAH (product formed) using a standard curve set up under similar conditions.

Step 3) Run dose response curve with sinefungin.

1) Titrate sinefungin using two- fold dilution by adding 7.5 µL aliquots to wells containing 7.5 µL enzyme buffer starting at 5000 µM and ending at 0 ng/µL (the maximum inhibitor concentration will be 2500 µM after addition of enzyme and substrate. We suggest running duplicates, with separate controls lacking the H4 peptide and SAM.

2) Add 5 µL of 8.7 ng/µL of PRMT1 at EC_{80} concentration, such that the final concentration of enzyme in the 15 µL reaction is at 2.9 ng/µL.

3) To one row of wells add 2.5 µL of substrate for final concentrations of 2 µM SAM and 10 µM peptide. Separate controls lacking SAM and H4 peptide are recommended. Incubate the plate at 30°C for two hours.

4) Add 2.5 µL of Stop Buffer A followed by 2.5 µL of detection mix.

5) Mix the plate well, incubate for an hour and read the plate in an instrument that measures fluorescent polarization at 633 nm EXC and 670 nm EMS.

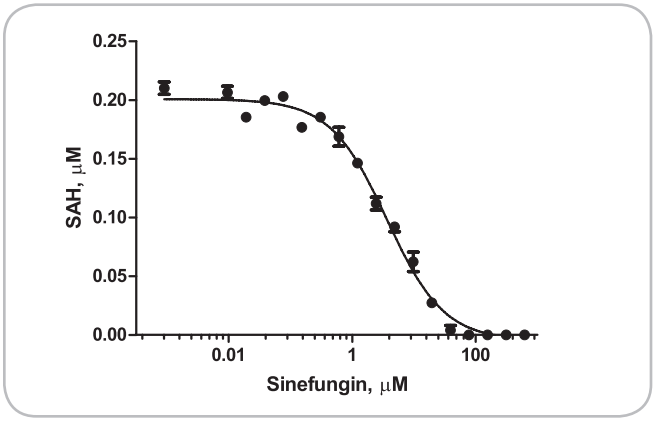

Figure 4. Sinefungin dose response curve. The polarization values were converted into product formed using a standard curve. The IC_{50} value was determined to be 4 µM.

Conclusions

This application note demonstrates a streamlined approach to develop a Transcreener EPIGEN Methyl transferase Assay for PRMT1. These steps can be followed to easily adapt the Transcreener EPIGEN Assay for doing high throughput screening with PRMT1.

References & Notes

1. Klink TA, Staeben M, Twesten K, Kopp AL, Kumar M, Schall Dunn R, Pinchard CA, Kleman-Leyer KM, Klumpp M, Lowery RG: Development and Validation of a Generic Fluorescent Methyltransferase Activity Assay Based on the Transcreener AMP/GMP Assay. J Biomol Screen 2012 Jan;17(1):59-70.

2. Makoto Tachibana, Kenji Sugimoto, Tatsunobu Fukushima and Yoichi Shinkai: PRMT 3, a Type I Protein Arginine N-Methyltransferase That Differs from PRMT1 in Its Oligomerization, Subcellular Localization, Substrate Specificity, and Regulation. J Biol Chem 1998 Jul;273 (27): 16935–16945.

Additional Information

Ordering Information

Please visit www.bellbrooklabs.com or contact BellBrook Labs for pricing for the Transcreener® Assays. Custom quotes are available for bulk orders.

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