

Application Note

Detection of HMT SET8 with the Transcreener® EPIGEN Methyltransferase Assay

This Application note will serve as a guide for using the Transcreener® EPIGEN Methyltransferase Assay to detect the initial velocity enzyme activity of HMT SET8 with an assay window suitable for inhibitor screening and dose response measurements. It should be used as an adjunct to the Transcreener EPIGEN Methyltransferase Assay.

Histone Methyltransferase SET8

SET8 catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the amino function of L-lysine residues (mono-, di- and trimethylation), especially of lysine-20, of histone H4². SET8 is essential for chromosome condensation, entry into mitosis and maintaining genomic stability. There is also evidence suggesting that SET8 may be a promising target for anti-cancer therapy. Human recombinant SET8 (residues 190-352; Genbank Accession # NM_020382) expressed as an N-terminal HIS 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.

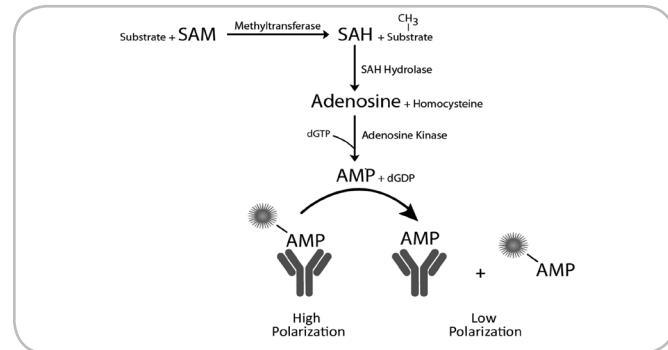


Figure 1. Transcreener Epigen methyltransferase assay principle: SAH produced in a methyltransferase reaction is converted to AMP in two sequential enzymatic steps. AMP is detected using a competitive fluorescence polarization immunoassay.



Summary of protocol

Step 1) Determine the optimal AMP²/GMP² antibody concentration for the SET8 enzyme reaction.

Step 2) Perform a SET8 titration to determine the amount required to produce a good assay window.

Materials and Methods

Instrument: Microplate Reader capable of measuring Fluorescence Polarization.

Microplates: Corning® 384 Well Low Volume Black Round Bottom PS NBSTM 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
SET8 (Reaction Biology)	HMT-11-118
H4 peptide (15-24) (Cayman Chemical)	10380

HMT Enzyme Buffer: 50 mM Tris-HCl (pH 8.5), 0.1 M NaCl, 4 mM DTT, 5 mM MgCl₂ and 0.01% Triton X 100.

Step 1) Determine the optimal AMP²/GMP² antibody concentration for the SET8 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; 3 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 substrate to product).

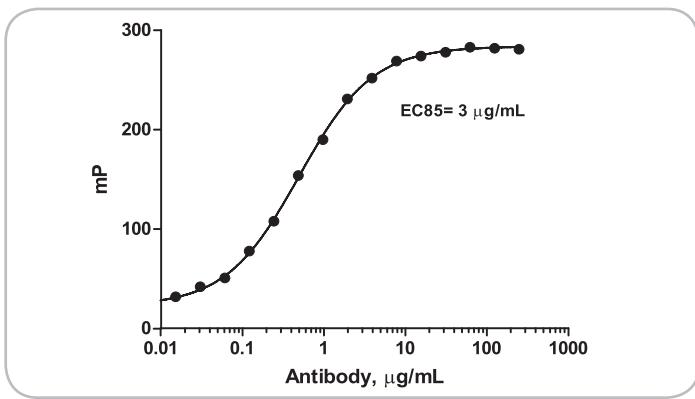


Figure 2. AMP²/GMP² antibody titration in the presence of 2 μM SAM and 10 μM H4 (15-24) peptide. An optimal concentration of 3 $\mu\text{g/mL}$ was determined based on this titration.

Step 2) Perform a SET8 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 SET8 using two-fold dilutions by adding 7.5 μL aliquots to wells containing 7.5 μL enzyme buffer starting at 12.5 ng/ μL and ending at 0 ng/ μL . (the maximum enzyme concentration will be 6.25 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 (15-24) 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 instructions 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, 3 $\mu\text{g}/\text{mL}$ of AMP² antibody, 0.125X cofactor, 0.125X detection buffer, 2 $\mu\text{g}/\text{mL}$ coupling enzyme 1 and 1 $\mu\text{g}/\text{mL}$ 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.

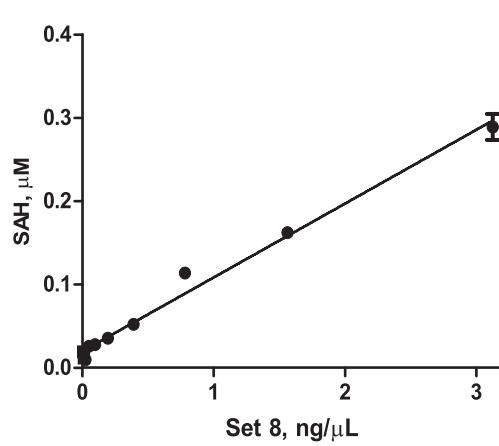


Figure 4. Linear relationship between Set8 concentration and SAH formation. The polarization values were converted into SAH (product formed) using a standard curve set up under similar conditions.

Conclusions

This application note demonstrates a streamlined approach to develop a Transcreener EPIGEN Methyl transferase Assay for SET8. These steps can be followed to easily adapt the Transcreener EPIGEN Assay for doing high throughput screening with SET8.

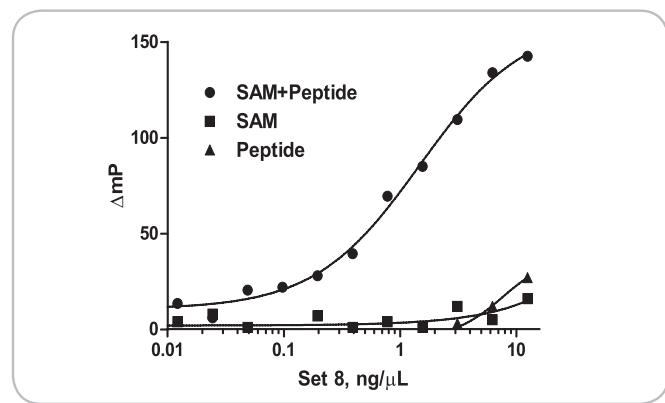


Figure 3. SET8 enzyme titration in the presence of 2 μM SAM and 10 μM H4 (15-24) peptide. A concentration (EC₅₀) of 3 ng/ μL was determined optimal based on this titration.

References & Notes

1. Klink TA, Staeben M, Tweten 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. Jean-Francois Couture, Evys Collazo, Joseph S Brunelle and Raymond C. Trievle: Structural and Functional Analysis of SET8, a Histone H4 Lys 20 Methyltransferase. *Genes & Dev* 2005 Jun 2;19: 1455-1465.

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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Transcreener® AMP ² /GMP ² Assay	3015-1K

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The Transcreener® product line is the subject of U.S. Patent No. 7,332,278, 7,355,010 and 7,378,505 issued. U.S. Patent Application Nos. 11/353,500, 11/958,515 and 11/958,965, U.S. Divisional Application 12/029,932, and foreign equivalents licensed to BellBrook Labs.