Profiling MT Inhibitors with Transzyme™ Methyltransferase Assay Kits

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Abstract

Methyltransferases are challenging targets from an assay development standpoint because they have low activity, high SAM affinities, and complex acceptor substrate requirements. Profiling inhibitor selectivity across several isoforms is especially challenging as it requires a combination of high sensitivity and the ability to accommodate diverse substrates and widely variable reaction conditions. The Transcreener® EPIGEN Methyltransferase Assay was developed to meet these needs. It is a universal, mix-and-read assay method with a far red fluorescence polarization readout that is capable of detecting SAH at low nanomolar concentrations. To accelerate MT screening efforts and streamline selectivity profiling we are collaborating with Reaction Biology, a supplier of high quality purified MT enzymes and substrates, to develop turn-key assay kits containing enzymes, substrates and Transcreener EPIGEN detection reagents. The purified enzymes in the Transzyme MT Assay kits have been calibrated to generate an HTS-quality assay window (Z'>0.6) under initial velocity conditions (<20% SAM consumption). We will present results on development of Transzyme MT Assay kits for several protein and DNA methyltransferases including Dot1L, NSD2 and PRMT1 and 3 and their use in selective profiling of MT-focused inhibitor library.

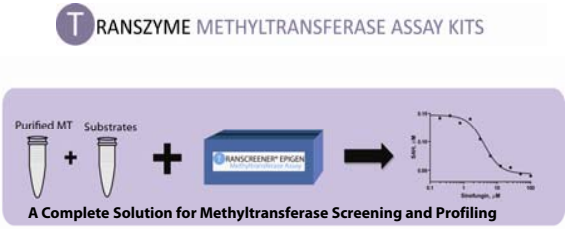


Figure 1. The Transzyme Methyltransferase Assay kits combine Transcreener® EPIGEN Methyltransferase Assay detection reagents with purified and validated MT enzymes from Reaction Biology and the optimal substrates. The kits are designed to allow investigators to begin screening or run dose response experiments without any assay development or pilot experiments. Enzyme and detection reagents are pre-calibrated to produce outstanding assay windows under initial verlocity (low substrate) conditions, insuring naccurate kinetics and inhibitor potency measurements.

Universal, HTS-proven Methyltransferase Detection in a Mix-and Read Format

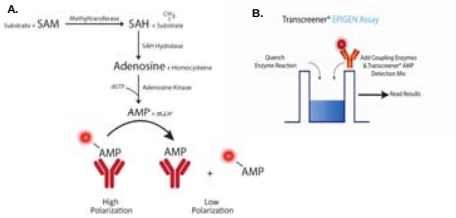
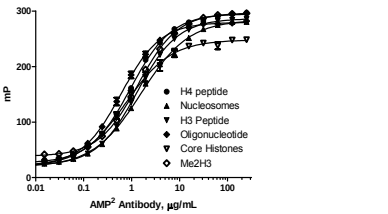


Figure 2. The Transcreener EPIGEN Methyltransferase Assay. A. The SAH produced in a methyltransferase reaction is converted to AMP in two enzymatic steps. The AMP is detected using the extensively validated Transcreener® AMP/GMP Assay, a competitive immunoassay, with a far red fluorescence polarization readout. B. The assays are a mix-and-read, homogenous format and can be run in stop time or continuous mode in either 384 or 1536 well plates.

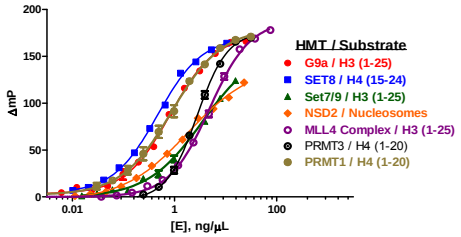
Precalibration of Detection Reagents for HTS Quality Assay Windows



	H4 peptide	Nucleosomes	H3 Peptide	Oligonucleotide	Core Histones	Me2H3
EC_{85}	5.197	11.55	7.089	3.111	4.924	7.058

Figure 3. Optimization of AMP² antibody concentration for use with different methyltransferase acceptor substrates. Tracer (4 nM) was titrated with AMP antibody in the presence of 2 µM SAM and the indicated substrate at its optimal concentration. The antibody concentration resulting in 85% saturation (EC_{85}) will yield a good assay window (> 100mP) for detection of initial velocity levels of SAH production. This information is provided with each of the Transzyme kits.

Precalibration of Enzymes for Initial Velocity Detection



	G9a	SET8	Set7/9	NSD2	MLL4 Complex	PRMT3	PRMT1
EC_{80}	3.375	1.89	19.58	14	17.1	7.155	4.208

Figure 4. Methyltransferase Enzyme Titration: Enzymes were titrated in 15 µL reactions in the presence of 2 µM SAM and substrate at appropriate concentration. The EC_{80}, which reflects 10-15% SAM conversion, is determined for each batch of enzyme used in a Transzyme kit and indicated on the Certificate of Analysis. Using the precalibrated EC_{85} concentration of enzyme will provide an outstanding assay window and insure initial velocity conditions, eliminating the need to use precious enzyme for time consuming assay development.

Linear Kinetics and 48 Hour Signal Stability

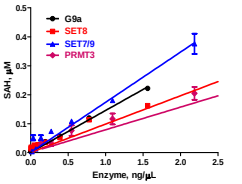


Figure 5. Tranzyme assay kits follow Michaelis Menten parameters. The linear correlation between the enzyme concentration and product formation demonstrates initial velocity conditions and adherence to Michaelis-Menten parameters.

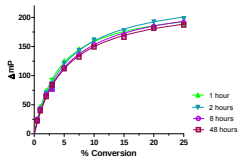


Figure 6. Stability of Assay Signal. A standard curve for conversion of SAM to SAH, starting at 2 µM SAM, was developed and the plate was read at intervals. The outstanding signal stability provides flexibility for automated protocols with large numbers of plates.

HTS Quality Performance

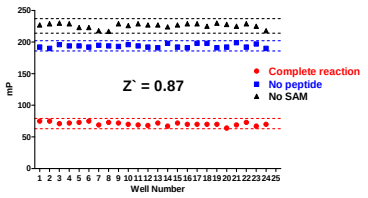


Figure 7. Z`-factor Determination for PRMT3: The PRMT3 Transzyme kit was used to determine Z' (n = 16) in a 384 well format, and read in a Tecan Safire plate reader in the FP mode at wavelengths EX-620 nm and EM-680 nm. The Z' of 0.87 and assay window of > 100mP reflects the excellent performance of the Transzyme kits using the precalibrated reagent quantities (3 ng/ µL of PRMT3, 10 µM H4 peptide, 2 µM SAM).

Methyltransferase Inhibitor Profiling with No Assay Development

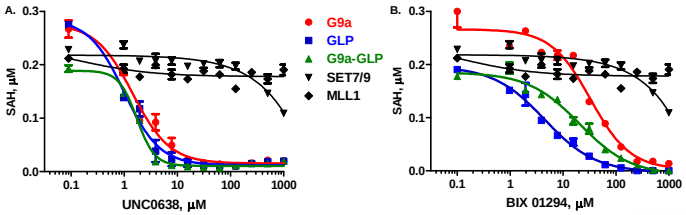


Figure 8. Dose Response Curves HMT G9a Specific Probes. Inhibitors UNC0638 (A) and BIX 01294 (B) (both from Sigma) were serially diluted and pre-incubated with EC_{80} concentrations of respective enzymes followed by addition of precalibrated substrates, stop mix, and detection reagents as described in the corresponding Transzyme kit instructions (C). Plates were read in a Tecan Safire at 620 EX and 670 EM in the FP mode.

Conclusions

- ***Transzyme kits include everything needed to begin screening or profiling your target HMT, including enzymes, substrates and detection reagents.***
- ***Eliminates assay development: Precalibrated enzymes, substrates and detection reagents deliver outstanding assay windows (Z' > 0.7) under initial velocity conditions with no assay development or pilot experiments.***
- ***HTS-Ready: Transcreener® HTS assay technology has been validated in over 50 million wells of screening.***

Transzyme MT Assay Kit	Catalog #	Quantity
DNMT1	9002	200-1000 Assays [384 well format]
HMT DOT1L	9014	200-1000 Assays [384 well format]
HMT G9a	9008	200-1000 Assays [384 well format]
HMT MLL4	9010	200-1000 Assays [384 well format]
HMT NSD2	9012	200-1000 Assays [384 well format]
HMT PRMT1	9014	200-1000 Assays [384 well format]
HMT PRMT3	9016	200-1000 Assays [384 well format]
HMT SET7/9	9018	200-1000 Assays [384 well format]
HMT SET8	9020	200-1000 Assays [384 well format]
HMT SUV39H1	9022	200-1000 Assays [384 well format]

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