Interrogating Chromatin Modifying Enzymes: Methyltransferases, Acetyltransferases, Kinases and SUMO ligases Using Transcreener® Assays

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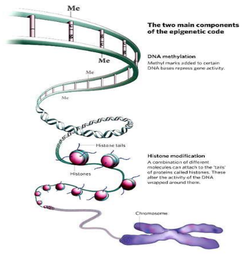
Overview

Biochemical HTS assays are a critical component of epigenetic drug discovery programs focused on the enzymes that catalyze posttranslational chromatin modifications; e.g., methyltransferases, acetyltransferases, kinases. Assembling high quality assay reagents and testing them with enzymes can consume a significant amount of time and resources. The shift toward screening focused libraries against multiple targets in parallel can add to the assay development burden substantially. Universal assay methods that detect the nucleotide by-products of chromatin modification reactions can simplify assay development substantially compared with methods that rely on detection of specific modification events. Transcreener is a highly validated, homogenous HTS platform that relies on fluorescence based detection of nucleotides, including ADP and AMP, and we have extended the detection capabilities to SAH and CoA using coupling enzymes. Here we describe how these Transcreener assays allow robust, initial velocity detection of four different types of chromatin modifying enzymes:methyltransferases, acetyltransferases, kinases, and SUMO ligases. The uniform design and readout of the Transcreener assays facilitates assay development and simplifies comparative data analysis for multiple epigenetic enzyme targets in parallel and provide a robust, cost-effective, HTS-proven platform for accurately measuring epigenetic enzyme initial velocity.

Chromatin Modifications



A. Modification at the DNA level

1. Cytosine Methylation

B. Histone modification - the Histone Code

1. Histone Acetylation
2. Histone Methylation
3. Histone Phosphorylation
4. Histone Ubiquitination
5. Histone Sumoylation

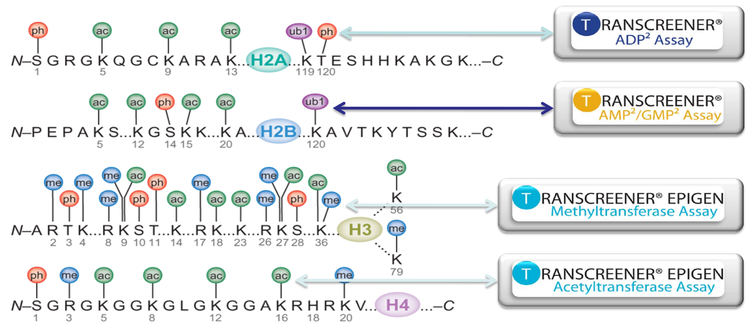


Figure 1. Illustration of nucleosome models and major posttranslational modifications which play essential roles in gene expression and regulation and disease processes. Diagram also shows the different Transcreener assays that could be used to study these epigenetic modifications.

Histone & Peptide Phosphorylation by PKA

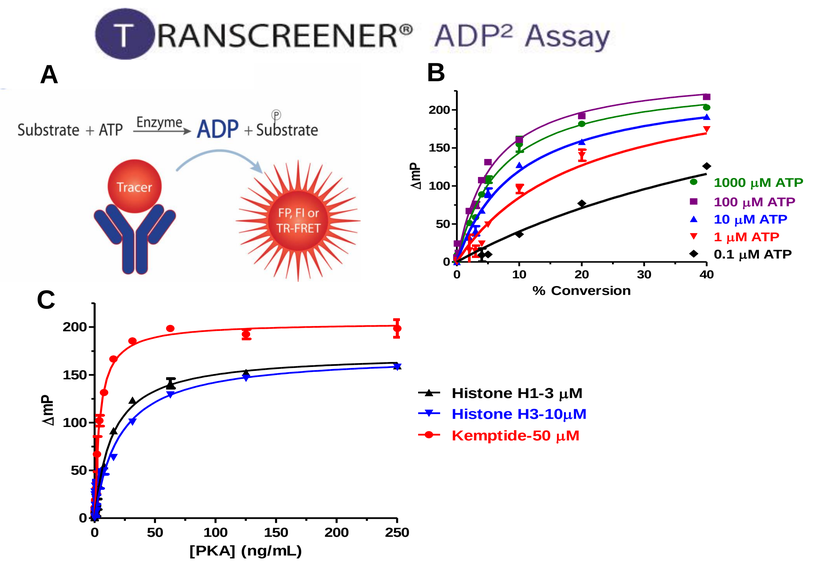
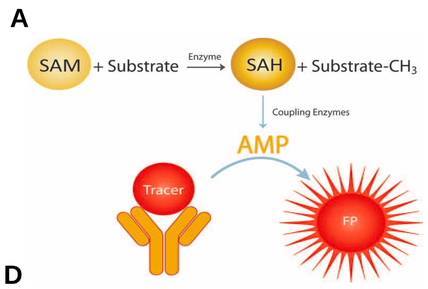
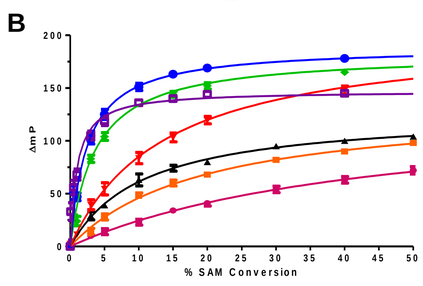


Figure 2. A) Transcreener assay platform is a single step homogenous immunoassay that allows the direct detection of ADP. The detection mix comprises of a far red tracer and a highly selective monoclonal antibody that can distinguish between nucleotides on the basis of a single phosphate group. B) Shows 0.1-1000 µM ATP/ ADP standard curve mimicking an enzyme reaction. C) PKA kinase was titrated in the presence of peptide (3-10 µM) or native protein substrate (50 µM) and allowed to incubate fro 1h at 25°C before adding an equal amount of Transcreener ADP² FP Stop & Detect reagents. Signal was read after 60 minutes equilibration time.

Monitoring Multiple Methyltransferase Activities with One Assay

TRANSCREENER® EPIGEN Methyltransferase Assay





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% Conversion	0.1 µM	0.25 µM	0.5 µM	1 µM	5 µM	10 µM	50 µM
30	0.67	0.75	0.82				
20	0.60	0.71	0.79	0.90	0.85	0.90	0.85
15	0.52	0.63	0.74	0.83	0.83	0.90	0.84
10	0.23	0.54	0.55	0.75	0.83	0.86	0.83
5		0.12	0.55	0.61	0.83	0.80	0.73
3			0.12	0.51	0.75	0.77	0.70
1				0.22	0.33	0.53	0.50
0.5					0.31	0.41	0.31

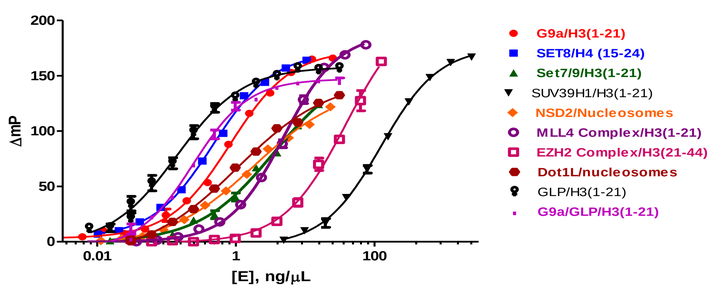


Figure 3. A) SAH produced in a methyltransferase reaction is converted to AMP in two sequential enzymatic steps. AMP is detected using Transcreener AMP²/GMP² FP Assay. B) Standard curves mimicking MT reactions (formation of SAH from SAM) show >100 mP signal at less than 20% conversion over the full range of initial SAM concentrations tested. C) Table of Z' values for various SAM concentrations at different % conversions, Z' values >0.5 are shown in green. D) Methyltransferases were titrated in 15 µL reactions in the presence of 2 µM SAM and appropriate concentration of substrate. After a 2h incubation at 30°C Transcreener EPIGEN Methyltransferase FP assays reagents were added to stop the reaction and to detect the SAH product .

Histone Acetylation by pCAF Acetyltransferase

TRANSCREENER® EPIGEN Acetyltransferase Assay

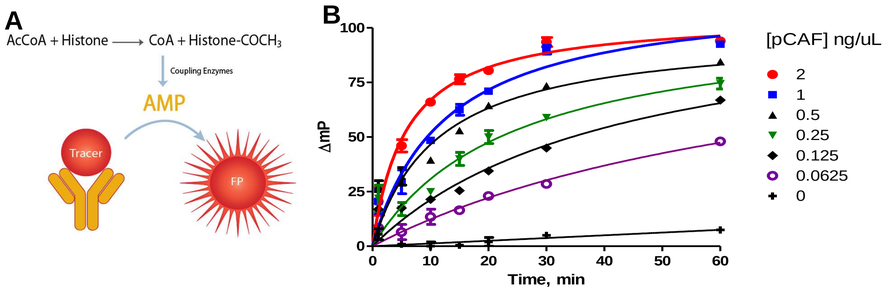
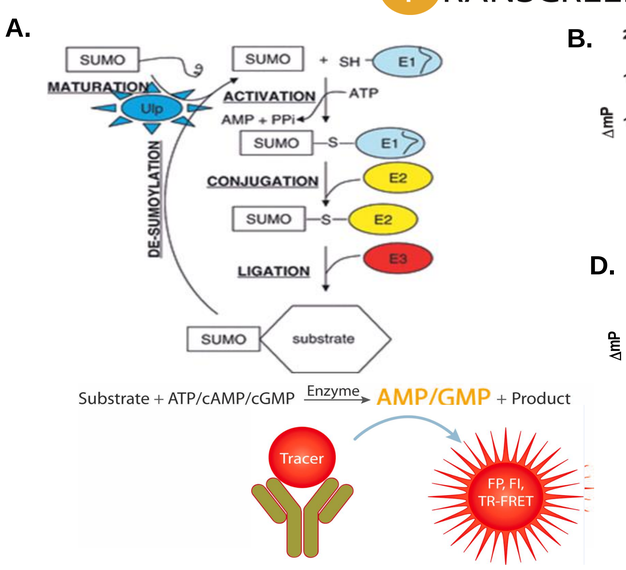
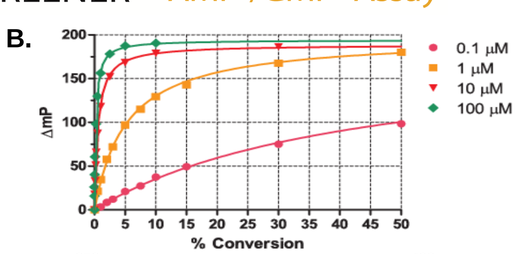


Figure 4. A) CoA produced in a acetylltransferase reaction is converted to AMP in one enzymatic step. AMP is detected using Transcreener AMP²/GMP² FP Assay. B) Enzymatic progress curves were performed by incubating pCAF acetyltransferase with 10 µM H3 peptide and 100 µM Acetyl CoA in 10 µL volume and were stopped by the addition of 10 µL Transcreener AMP²/GMP² FP detection mix containing antibody and tracer, the coupling enzyme system, and 50 µM Anacardic Acid at the indicated time points. Signal was read after 60 minutes.

Studying AMP Flux through the Histone Sumoylation/Ubiquitination Pathway

TRANSCREENER® AMP²/GMP² Assay





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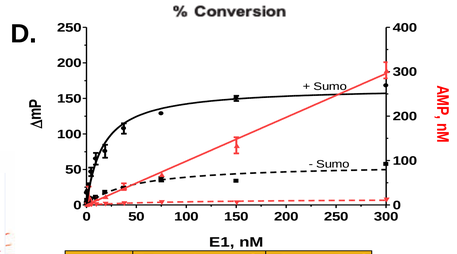
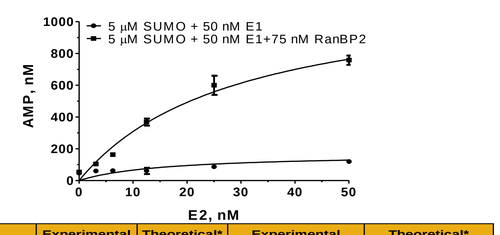
% Conversion	0.1 µM	1 µM	10 µM	100 µM
30	0.76	0.90	0.95	
10	0.66	0.88	0.93	0.92
5	0.50	0.82	0.93	0.91
1		0.58	0.86	0.85
0.5		0.41	0.81	0.78
0.1			0.55	0.54



E1, nM	Experimental AMP, nM	Theoretical AMP, nM
150	134 (±8.6)	150
75	69 (±4.2)	75
37.5	41 (±7.4)	37.5
18.7	19 (±4.1)	18.7
9.3	8 (±3.1)	9.3



E2, nM	Experimental + 50 nM E1 AMP, nM	Theoretical* + 50 nM E1 AMP, nM	Experimental + 50 nM E1 + 75 nM E3 AMP, nM	Theoretical* +50 nM E1+ 75 nM E3 AMP, nM
50	119 (±7.2)	100	758 (±4.8)	175
25	86 (±4.3)	75	600 (±6.2)	150
12.5	61 (±5.4)	62.5	368 (±7.1)	137.5
0	47 (±3.4)	50	53 (±1.4)	50

Figure 5. A) Schematic of the sumoylation pathway showing AMP production during E1 activation. AMP flux thru the pathway can be monitored with the Transcreener AMP²/GMP² Assay. **B)** Substrate standard curve demonstrating robust signal (FP) at low percent conversions with four different concentrations of initial ATP. C) Table showing the Z' values obtained from n=24 replicates for four different concentrations of ATP/AMP standard curves. D) AMP Formation is SUMO-dependent and stoichiometric with E1. Reactions were performed with 5 µM ATP + 5 µM SUMO and were allowed to go to completion with overnight incubation. **E)** AMP formation is also dependent upon stoichiometric amounts of E2. UbcH2 enzyme (E2) was titrated across 50 nM UBE1 (E1) and 50 nM UBE1 (E1) with 75 nM E3. The theoretical and experimental quantities of AMP produced are shown in the table. E3 undergoes poly-sumolylation and therefore leads to more AMP formation.

Summary

- BellBrook Labs offers a suite of homogenous, fluorescence-based, nucleotide detection assays that can be used to screen and profile a variety of Chromatin modifying enzymes including methytransferases, acetyltransferases, kinases, and sumo/ubiquitin ligases.
- The universal nature of these Transcreener HTS assays (detection of the invariant by-product of the reaction) enables simple monitoring of multiple enzyme activities in parallel.
- Sensitive detection and robust signal (FP, FI, or TR-FRET) provide accurate measurements of enzyme initial velocity.

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