Development and Validation of a Generic Fluorescent Activity Assay for Acetyltransferases Based on the Transcreener® AMP/GMP Assay

Meera Kumar* and Robert G. Lowery
BellBrook Labs LLC, Madison, WI, USA

Abstract

Acetylation is a ubiquitous covalent modification used to control the function of diverse biomolecules including hormones, neurotransmitters, xenobiotics, and proteins. Histone methyltransferases (HATs), common components of nuclear receptor coactivator complexes, are currently of interest as drug targets because of their role in epigenetic regulation. However, most HAT assay methods are either not amenable to a HTS environment or are applicable to a limited number of enzymes. We developed a generic HAT assay method using fluorescent immunodetection of AMP, which is formed from the HAT reaction product coenzyme A by a coupling enzyme. In this poster we present proof of concept for the assay, including dependence on enzyme, time and substrates and inhibitor dose response using the p300/CBP-associated acetyltransferase pCAF and a histone H3 peptide substrate as a model system. The results indicate that the assay is well suited for HAT enzyme initial velocity detection at physiological AcCoA concentrations with total polarization shifts of 70-140 mP and Z' values of 0.6 to 0.7. By combining a novel enzymatic coupling step with the well characterized Transcreener® AMP/GMP assay, we have developed a robust HTS assay for HATs, which should be broadly applicable to other types of acetyltransferases as well.

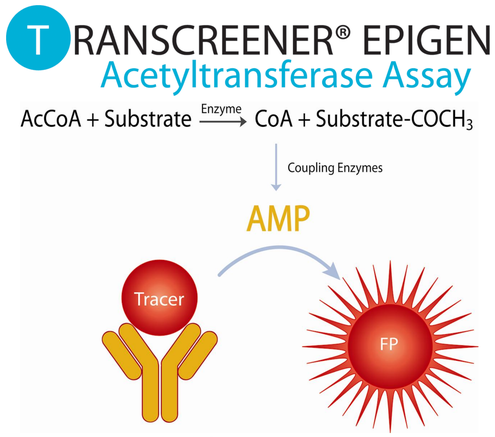


Figure 1. Assay Principle. CoA produced by HAT is enzymatically converted to AMP, which is detected using the Transcreener ® AMP/GMP Assay.

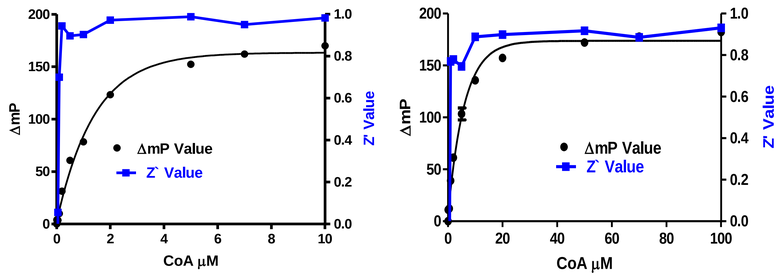


Figure 2. Standard Curves Mimicking Conversion of Acetyl CoA to CoA
A) Initial CoA concentration was 10 µM; B) Initial CoA concentration was 100 µM. For both curves, CoA was added and Acetyl CoA decreased proportionately keeping the total coenzyme concentration constant. 20 µL reactions contained 2 nM AMP/GMP tracer, 15 µg/mL and 45 µg/mL of AMP²/GMP² antibody respectively, and coupling enzyme/substrate mixture. The Z` at 10% conversion is > 0.7 at both 10 and 100 µM standard curves with ΔmP values of 70 and 135, respectively.

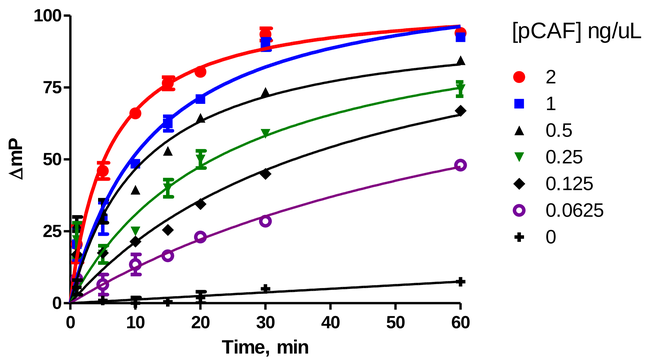


Figure 3. Enzyme and Time Dependence: pCAF. Enzymatic progress curves were performed by incubating pCAF enzyme (2 ng/µL to 0.0625 ng/µL) with 10 µM H3 peptide and 100 µM Acetyl CoA in 10 µL volume and were stopped by the addition of 10 µL detection mix containing AMP²/GMP² antibody and tracer, the coupling enzyme system, and 50 µM Anacardic Acid at the indicated time points. Signal was read after 60 minutes. A 60 minute enzyme reaction with 0.4 ng/µL of pCAF was selected for all subsequent experiments. Controls lacked the H3 peptide.

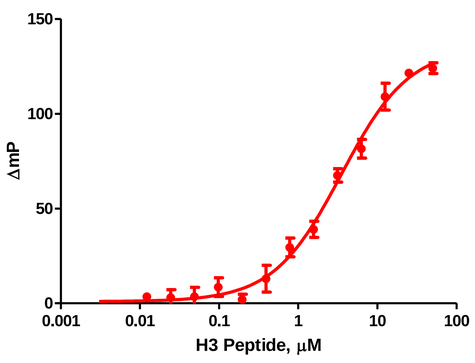
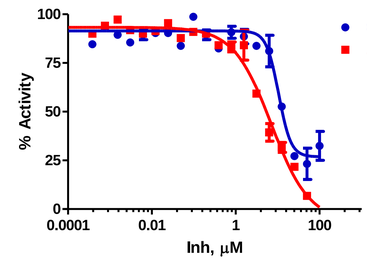


Figure 4. Substrate Dependence. Histone H3 peptide was titrated in pCAF reactions in the presence and absence of 100 µM Acetyl CoA. 10 µL reactions containing 0.4 ng/µL of pCAF were run at room temperature for 60 min followed by addition of detection and quench reagents. Signal was read after a 60 min. equilibration period. Controls lacked Acetyl CoA.



	Garcinol	Anacardic Acid
IC50 (µM)	10.53	7.07

Figure 5. Determining IC_{50} Values. Dose response curves of representative inhibitors of pCAF generated using the EC_{80} concentration of pCAF (0.4 ng/µL) in the presence of 100 µM Acetyl CoA and 10 µM H3 peptide are shown. Reactions were run as described for Figure 3. Controls lacked the H3 Peptide.

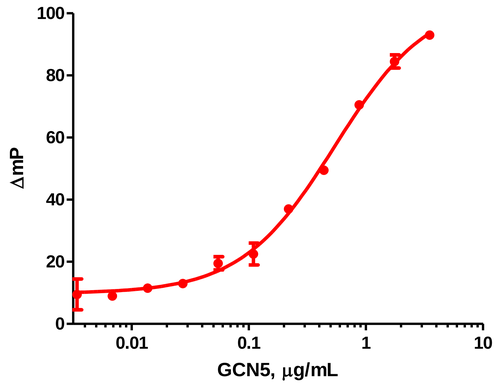


Figure 6. Enzyme Dependence: GCN5. GCN5 was titrated into reactions containing 10 µM Histone H3 peptide and 100 µM AcCoA in a reaction volume of 10 µL. Reactions were processed as described in Figure 3 and signal was read after 60 minutes. Controls lacked the H3 peptide.

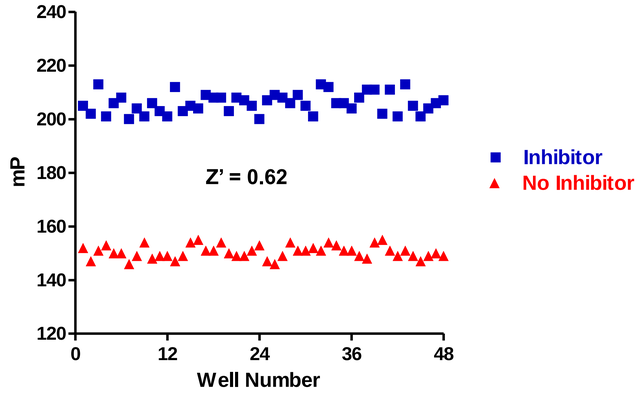


Figure 7. Assay Window. pCAF (0.4ng/µL) with 10 µM Histone H3 peptide was pre-incubated with and without 30 µM Anacardic Acid for 15 minutes in a reaction volume of 7.5 µL. Enzymatic reactions were initiated by the addition of 2.5 µL of 100 µM Acetyl CoA. Reactions were processed as described for Figure 3. N = 48. Z` value was 0.62.

Conclusions

- Coenzyme A produced in HAT reactions can be enzymatic converted to AMP to enable detection with the Transcreener AMP/GMP Assay.
- Formation of AMP was shown to be dependent on HAT enzyme, Acetyl Coenzyme A and Histone H3 acceptor peptide.
- The Transcreener Epigen HAT Assay is capable of detecting initial velocity levels of CoA formation with an assay window suitable for HTS (ΔmP = 70-140 mP, Z' ≥ 0.6).
- The IC_{50} values of two well known inhibitors, Anacardic Acid and Garcinol were determined to be 7 and 10 µM respectively with pCAF.

BellBrook Labs, 5500 Nobel Drive, Suite 250, Madison, WI 53711
866.313.7881 or 608.443.2400