Application Note

Inhibitor Dose Response Measurement with the Transzyme Methyltransferase Assay Kit

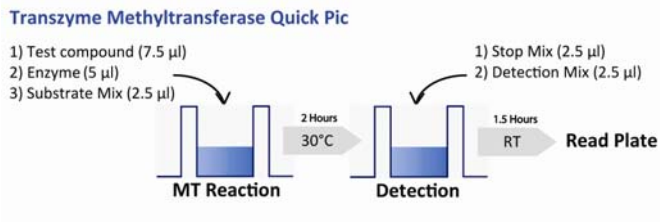
Meera Kumar
BellBrook Labs, Madison-WI

Introduction

This Application Note describes how to generate a dose response curve for an inhibitor using a Transzyme Methyltransferase Assay kit. The ultimate goal of biochemical HTS is to identify "hit" compounds against the enzyme being targeted. Once a hit is identified, it is analyzed in more detail to determine its potency as an inhibitor. Potency is usually expressed as the half maximal inhibitory concentration, or IC_{50} value, which is generally determined by constructing a dose-response curve.

The Transzyme Methyltransferase kit offers a complete HTS assay solution, with a validated purified enzyme, an optimal acceptor substrate and enzyme buffer and predetermined reaction conditions that yield a Z' of at least 0.6 in BellBrook's laboratories. The kit also provides the optimal enzyme concentration (EC_{80}) required for running dose response curves. The kits remove essentially all of the assay development normally required for establishing an HTS assay. However, differences in instrumentation and other uncontrolled variables can affect assay performance, and it is important to validate assay quality in the end user's laboratory.

Shown below is a general protocol for setting up an experiment to run dose response curves. As an example we show here how to run an inhibitor titration using Sinefungin as an example for HMT SET7/9 at its EC_{80} concentration to determine the IC_{50} value.



Methods

Transzyme reagent preparation and assay protocol are as described in the Transzyme Quick Guide for the specific MT being used. Briefly:

MT Reaction Reagents:

1) Sinefungin/Compound of choice: Dilute Sinefungin to be tested at 2X desired concentration in 1X HMT Enzyme Buffer.

2) Enzyme: Dilute SET7/9 to 3X EC80 concentration in 1X HMT Enzyme Buffer. Please see Certificate of Analysis for SET7/9 *Enzyme EC_{80} concentration, as this information is lot-specific.* Mix gently (do not vortex) and store on ice.

3) Substrate Mix: Dilute SAM to 12 µM and H3 Peptide (1-25) acceptor to 60 µM in 1X HMT Enzyme Buffer.

4) Control Substrate Mixes: a) Minus peptide - Dilute SAM to 12 µM in 1X HMT Enzyme Buffer. b) Minus SAM - Dilute H3 (1-25) peptide to 60 µM in 1X HMT Enzyme Buffer.

Detection Reagents:

1) Stop Mix: Use undiluted

2) Detection Mix: 32 nM AMP^2/GMP^2 AlexaFluor® 633Tracer, 1X Cofactor, 1X Detection Buffer, 16 µg/mL Coupling Enzyme 1, 8 µg/mL Coupling Enzyme 2, 40 µg/mL of AMP^2/ GMP^2 Antibody (see Technical Manual for Transcreener EPIGEN Methyltransferase Assay).

Protocol:

- Add 7.5 µL of 1X HMT Enzyme Buffer to wells A-F (1-15).
- Add 15 µL of Sinefungin at 5000 µM to wells A1, B1, C1, D1, E1 and F1. Perform a serial titration by transferring 7.5 µL of Sinefungin from column 1 to column 2, mix well and transfer 7.5 µL from column 2 to 3 and so on, until column 14. Column 15 will be the control lacking inhibitor. All wells should have a final volume of 7.5 µL.
- Add 5.0 µL of SET7/9 Enzyme to wells A-F (1-15). Please treat the SET7/9 enzyme gently, as dilute enzymes are easily denatured!
- Add 2.5 µL of Substrate Mix to wells A1-B15. Addition of substrate initiates the SET7/9 reaction.
- Add 2.5 µL of Control Substrate Mixes - Minus SAM Mix to wells C1-D15 and Minus Peptide Mix to wells E1-F15; All wells should now have 15 µL reactions. Mix plate well and place at 30°C immediately after this addition for two hours.
- Mix again after adding Stop Mix. Detection Mix can be added at anytime within 30 minutes of stopping reactions.
- Plates can be read at any time from 1.5 to 16 hours after addition of Detection Mix

Sinefungin/Test Compound	Volume (µL)	Final Conc in 15 µL Enzyme reaction
10X HMT Buffer	12	1X
Sinefungin (20 mM)	30	2.5 mM
Water	78	
Total	120	

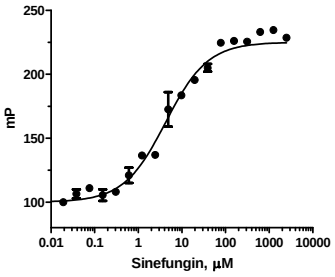
Enzyme (EC_{80}) Mix	Volume (µL)	Final Conc in 15 µL Enzyme reaction
10X HMT Buffer	60	1X
SET7/9 (510 ng/µL)	8	2.4 ng/µL
Water	532	
Total	600	

Substrate Mix and Controls	Substrate Mix (µL)	Minus Peptide (µL)	Minus SAM (µL)
10X HMT Buffer	20	20	20
SAM (5mM)	0.48	0.48	
H3 (1-25) Peptide (500 µM)	24		24
Water	155.52	179.52	156
Total	200	200	200

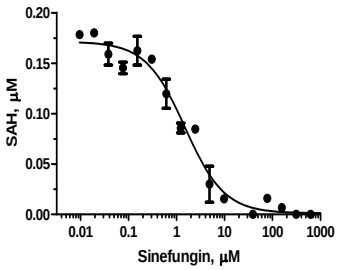
Data Analysis

The polarization values obtained from this experiment is fit using Graph Pad Prism Software. We recommend using the log [Inhibitor] versus response using the four parameter fit. One can use the IC_{50} value calculated from the raw polarization data for rank ordering and comparing the potencies of the compounds (Fig. 2a). However, for more accurate IC_{50} values it will be necessary to establish a standard curve to convert polarization values to SAH formed (see Transcreener EPIGEN MT Assay Technical Manual) and determine IC_{50} value from the converted data (Fig. 2b).

a)



b)



	Raw Data	Converted Data
$IC_{50\ (\mu M)}$	4.256	1.501

Figure 2: Shows IC_{50} determination using EC_{80} concentration of SET7/9 in the presence of 2 µM SAM and 10 µM H3 (1-25) peptide in a 15 µL enzyme reaction. The IC_{50} value was determined to be 4.2 µM using raw polarization units (a) versus 1.5 µM using the converted data (b).

Conclusions

We show a simple protocol for determining inhibitor potency for methyltransferases using the Transzyme Methyltransferase Assay.

Additional Information

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

Ordering Information

Phone Orders:
608.443.2400
866.3137881

Fax Orders:
608.441.2967

Email Orders:
info@bellbrooklabs.com

Technical Information

For technical information, please contact:

Meera Kumar, Applications Scientist
Tel: 608.443.2400
Toll-Free: 866.313.7881
Email: meera.kumar@bellbrooklabs.com.

BellBrook Labs

Toll-Free: 866.313.7881
www.bellbrooklabs.com