Application Note

Z' Determination with the Transzyme Methyltransferase Assay Kit

Meera Kumar
BellBrook Labs, Madison-WI

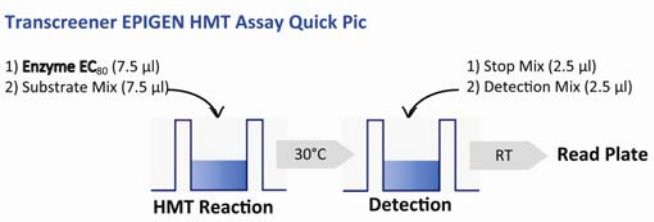
Introduction

This Application Note describes how to determine Z`, the statistical parameter used to measure HTS assay quality, using a Transzyme Methyltransferase Assay kit. The Z` was conceived in 1999 specifically to allow comparison of assays with different types of detection mechanisms (e.g. competitive binding vs. direct binding) and readouts (e.g. fluorescence polarization vs. TR-FRET). By taking into account both dynamic range and data variability at the high and low ranges of the assay, the Z' statistic provides a measure of what is of most interest when considering the suitability of an assay for HTS: the usable screening or assay window. It is a dimensionless coefficient for the quality of the screening window that is relevant for any assay, regardless of detection method or readout, without the intervention of test compounds. As a guideline, a Z' value of 0.5 or greater is generally considered to be indicative of a very good screening window for a biochemical assay.

$$Z' = 1 - \frac{3(\text{s.d. high control} + \text{s.d. low control})}{(\text{ave. high control} - \text{ave. low control})}$$

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 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 determine Z` value. As an example we have used PRMT3 as the HMT for Z` calculation using 24 replicates. For a robust Z` calculation we suggest running at least 8 replicates for each condition: complete reaction, minus peptide, and minus SAM.



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:

All MT reaction reagents are diluted in HMT Enzyme buffer (supplied at 10X) which should be at a 1X final concentration.

1) Enzyme EC_{80}: Dilute HMT PRMT3 to 2X final concentration in 1X HMT Enzyme Buffer. *Please see Certificate of Analysis for optimal HMT PRMT3 concentration, as this information is lot-specific.* Mix gently (*do not vortex*) and store on ice.
2) Substrate Mix: Dilute SAM to 4 µM and H4 (1-20) peptide to 20 µM in 1X HMT Enzyme Buffer.
3) Control Substrate Mixes: a) Minus Peptide: Dilute SAM to 4 µM in 1X HMT Enzyme Buffer. b) Minus SAM: Dilute H4 (1-20) peptide to 20 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 and 40 µg/mL AMP^2/ GMP^2 Antibody as indicated in the corresponding Quick Guide.

Protocol:

- Add 7.5 µL of PRMT3 Enzyme to wells A-C (1-24). Please treat the PRMT3 enzyme gently, as dilute enzymes are easily denatured!
- Add 7.5 µL of Substrate Mix to wells A1-A24. Addition of substrate initiates the PRMT3 reaction.
- Add 7.5 µL of Minus Peptide Mix to wells B1-B24 and the same amount of Minus SAM Mix to wells C1-C24; Mix plates 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

Enzyme (EC_{80}) Mix	Volume (µL)	Final Conc in 15 µL Enzyme reaction
10X HMT Buffer	65	1X
PRMT3 (1520 ng/µL)	3	3.5 ng/µL
Water	582	
Total	650	

Substrate Mix and Controls	SAM+ Peptide (µL)	Minus Peptide (µL)	Minus SAM (µL)
10X HMT Buffer	25	25	25
SAM (5mM)	0.2	0.2	
H4 (1-20) Peptide (500 µM)	10		10
Water	214.8	224.8	215
Total	250	250	250

Data Analysis

$$Z' = 1 - \frac{3(\text{Stdev (Minus SAM)} + \text{Stdev (SAM+Peptide)})}{(\text{Ave. (Minus SAM)} - \text{Ave. (SAM+Peptide)})}$$

or

$$Z' = 1 - \frac{3(\text{Stdev (Minus Peptide)} + \text{Stdev (SAM+Peptide)})}{(\text{Ave. (Minus Peptide)} - \text{Ave. (SAM+Peptide)})}$$

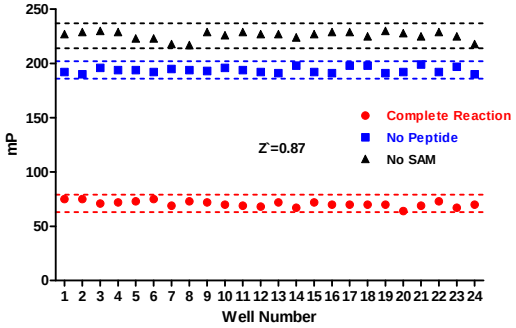


Figure 2: Shows Z' determination using EC_{80} concentration of PRMT3 in the presence of 2 µM SAM and 10 µM H4 (1-20) peptide in a 15 µL enzyme reaction. The Z` value was determined to be 0.87 using 24 replicates when determined using SAM or Peptide as high control.

Conclusions

A Z`>0.5 is typically considered a good assay. We show here that using Transcreener EPIGEN assay along with the purified enzyme and substrate pack ensures a Z`=0.8 demonstrating an outstanding assay window and a robust 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