Measuring Methyltransferase Activity Using the MTase-Glo™ Methyltransferase Assay and the GloMax® Discover System

Promega Corporation

Materials Required

- MTase-Glo™ Methyltransferase Assay (Cat.# V7601 and V7602)
- GloMax® Discover System (Cat.# GM3000)
- white, low volume 384-well assay plates (Corning Cat.# 4512)
- Nuclease-Free Water (Cat.# P1195)
- 4X Reaction Buffer: 80mM Tris buffer, pH 8.0; 200mM NaCl; 4mM EDTA; 12mM $MgCl_2$; 0.4mg/ml BSA; 4mM dithiothreitol (DTT)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *GloMax® Discover System Operating Manual* #TM397 and *MTase-Glo™ Assay Technical Manual* #TM453 are available at: **www.promega.com/protocols/**

The MTase-Glo™ Methyltransferase Assay is a bioluminescence-based assay that can be used to monitor the activities of methyltransferases (MTases) and their modulation by small molecules in high-throughput screening applications. The assay monitors formation of the reaction product S-adenosyl homocysteine (SAH) and can detect changes in activity of a broad range of methyltransferases, including DNA, protein, RNA and small molecule methyltransferases. The MTase-Glo™ Assay can be used for all classes of protein methyltransferases (lysine and arginine) and with different types of substrates (peptides, large proteins and even nucleosomes) to determine the specificity of these enzymes and their substrate requirements.

The MTase-Glo™ Methyltransferase Assay produces high signal-to-background ratios with a low coefficient of variation (CV), is robust (Z´ value > 0.7) and is compatible with 96-, 384-and 1,536-well plate formats. The assay can be used with a broad range of substrates to generate kinetic data and determine the mechanism of action of various methyltransferase modulators, and is unaffected by high substrate concentrations or substrate type (short versus long peptides).

After the methyltransferase reaction is complete, the MTase-Glo™ Reagent is added to convert SAH to ADP. Then the MTase-Glo™ Detection Solution is added to convert ADP to ATP, which is detected via a luciferase reaction. Luminescence is measured using a plate-reading luminometer and can be correlated to SAH concentration using an SAH standard curve. The half-life of the luminescent signal is greater than 4 hours. This extended signal half-life eliminates the need for luminometers with injectors and allows batch-mode processing of multiple plates.

Measuring the luminescence from the MTase-Glo™ Assay is easy on the GloMax® Discover System because the protocol comes preloaded on the instrument. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allows you to easily measure signals of varying intensities on the same plate. This Application Note describes the protocol to measure methyltransferase activity using the MTase-Glo™ Assay and GloMax® Discover System.

MTase-Glo™ Assay Protocol

For detailed instructions and assay notes for various assay volumes and plate formats, see the *MTase-Glo™ Methyltransferase Assay Technical Manual* #TM453. The following protocol is performed in 384-well low-volume plates.

1. Prepare SAH Standards containing 0–1µM SAH in 1X Reaction Buffer. Dispense 4µl of each SAH Standard into duplicate wells of a 384-well low-volume plate.
2. Gently mix thawed MTase-Glo™ Reagent; do not vortex. Prepare 5X MTase-Glo™ Reagent by mixing equal volume of 10X MTase-Glo™ Reagent with Nuclease-Free Water.
3. Add 1µl of 5X MTase-Glo™ Reagent to each well containing SAH Standards. Final volume is 5µl per each well.
4. Incubate the reaction at room temperature (22–25°C) for the 30 minutes.

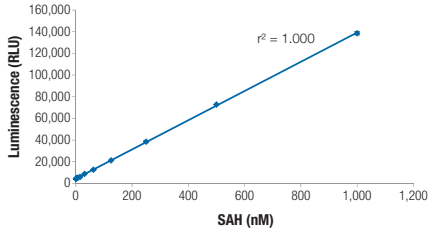
   Note: Store unused 10X MTase-Glo™ Reagent at –65°C. Alternatively, dispense the 10X MTase-Glo™ Reagent into single-use aliquots, and store at –65°C. Do not freeze diluted MTase-Glo™ Reagent.
5. Thaw MTase-Glo™ Detection Solution at room temperature, and gently mix by inversion. Do not vortex.

   Note: The remaining MTase-Glo™ Detection Solution can be stored long-term storage below –65°C. For short-term storage, store at –20°C.
6. Add 5µl of MTase-Glo™ Detection Solution to each well, mix briefly and incubate with shaking for 30 minutes at room temperature (22–25°C).
7. Measure luminescence on the GloMax® Discover System by selecting "MTase-Glo" from the list of preset protocols.

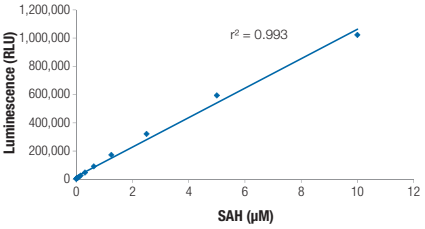
Conclusion

The GloMax® Discover can detect luminescence generated using the MTase-Glo™ Methyltransferase Assay as shown in Figure 1. The increase in light output correlates with the amount of SAH, the product of the methyltransferase reaction, and thus MTase activity.

A.



B.



C.

SAH (nM)	1,000	500	250	125	62.5	31.3	15.6	7.81	3.91	1.95	0.98	0
RLU	138,575	72,855	38,495	21,140	12,618	8,723	6,064	5,236	4,975	4,566	4,193	4,181
S:B	33.1	17.4	9.2	5.1	3.0	2.1	1.5	1.3	1.2	1.1	1.0	1.0
S:N	679	347	173	86	43	23	10	5	4	2	0	0

RLU: An average of light output from quadruplicates
STDEV: Standard deviation; Background STDEV is 97 RLU
S:B: Signal-to-background ratio; Mean Signal ÷ Mean Background
S:N: Signal-to-noise ratio; (Mean Signal – Mean Background) ÷ (Background STDEV)

13566MA

Figure 1. S-adenosyl-homocysteine (SAH) titration using the MTase-Glo™ Assay. SAH (0–1µM) was measured using the MTase-Glo™ Assay in solid white 384-well low-volume plates as described in the protocol. **Panels A and B**. Luminescence was recorded using the GloMax® Discover System within 30 minutes after adding the Detection Solution. Data were analyzed using Microsoft Excel® software and represented as mean ± standard error (n = 4). **Panel C.** Table showing the luminescence generated at various SAH concentrations and calculated signal-to-background (S:B) and signal-to-noise (S:N) ratios. Limit of detection is 3 × (S:N). Data were analyzed using Excel software and represented as mean ± standard error (n = 4).

The GloMax® Discover System

The GloMax® Discover System offers superior sensitivity and dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network.