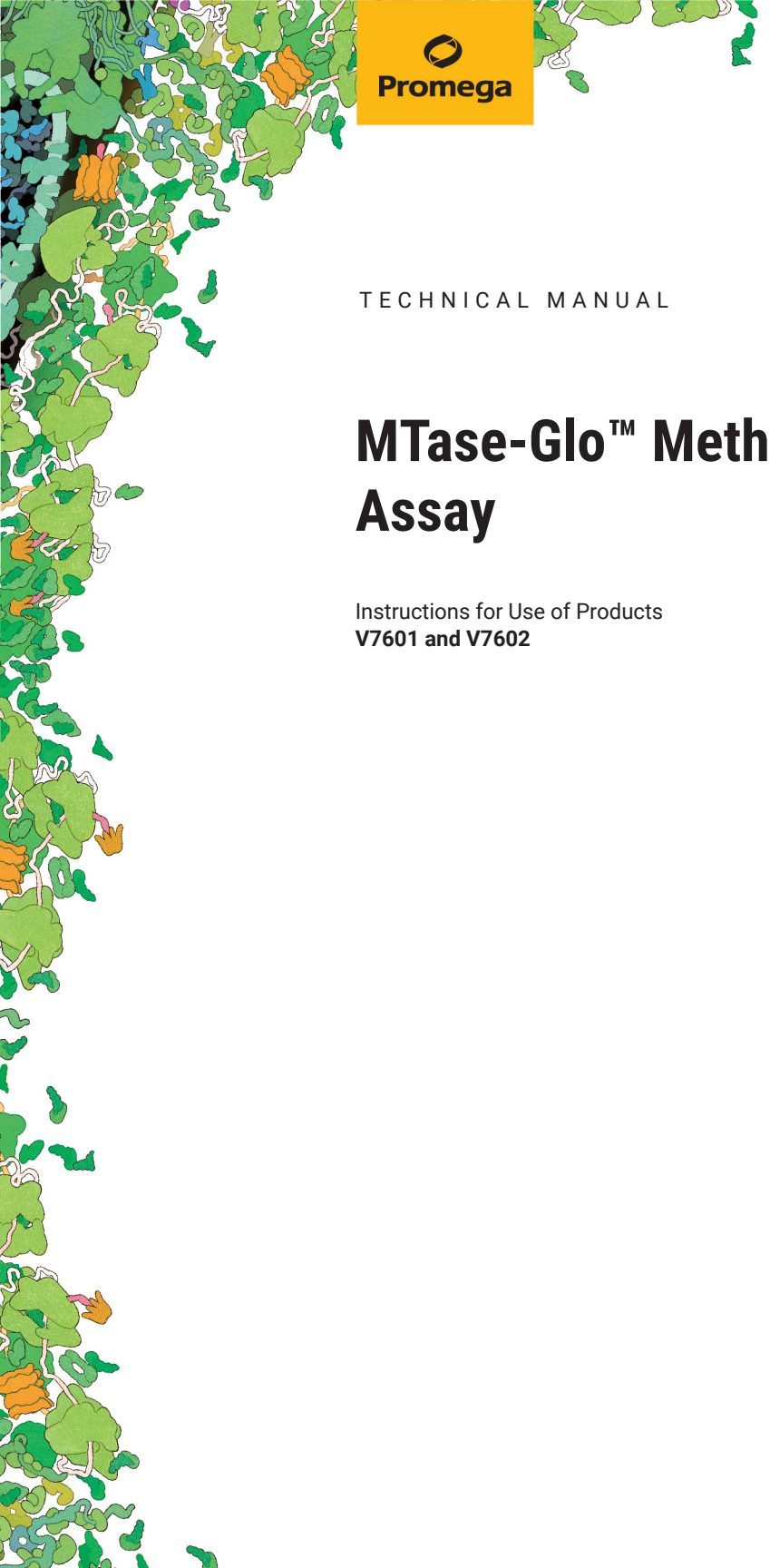

TECHNICAL MANUAL

MTase-Glo™ Methyltransferase Assay

Instructions for Use of Products
V7601 and V7602

Revised 3/24
TM453

Promega

MTase-Glo™ Methyltransferase Assay

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Post-translational, post-transcriptional and epigenetic modifications of proteins and nucleic acids are known to play major roles in influencing cell fate. Enzymes that catalyze modifications such as phosphorylation, acetylation and methylation have been identified as drug targets, and recent biochemical and biological data suggest that some of these enzymatic activities have pathogenic roles in cancer, inflammation and neurodegenerative diseases (1–5). Of these enzymes, methyltransferases are known to effect changes in cellular function and physiology by altering the epigenome and modulating the methylation status of nucleic acids and proteins. Thus, having a sensitive, universal methyltransferase assay is beneficial when testing small molecules to identify methyltransferase activators or inhibitors in drug discovery applications.

1. Description (continued)

The MTase-Glo™ Assay[a,b] 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-well, 384-well 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. The MTase-Glo™ Detection Solution is then added to convert ADP to ATP, which is detected via a luciferase reaction (Figure 1). Luminescence is measured using a plate-reading luminometer (Figure 2) and can be correlated to SAH concentration using an SAH standard curve (Figure 3). 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.

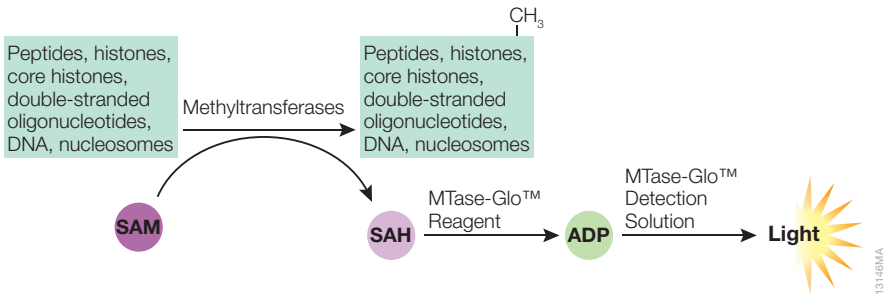

Figure 1. MTase-Glo™ Methyltransferase Assay. The substrate can be a peptide, histone, core histone (e.g., H3 or H4), double-stranded DNA oligonucleotide, DNA or nucleosome.

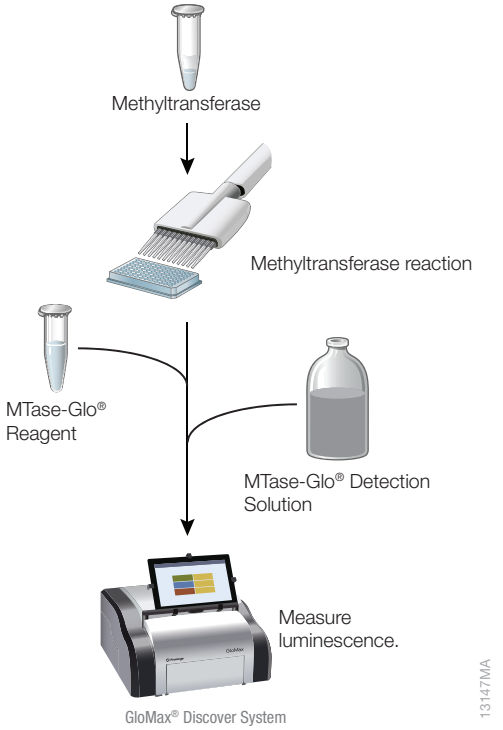

Figure 2. MTase-Glo™ Methyltransferase Assay protocol.

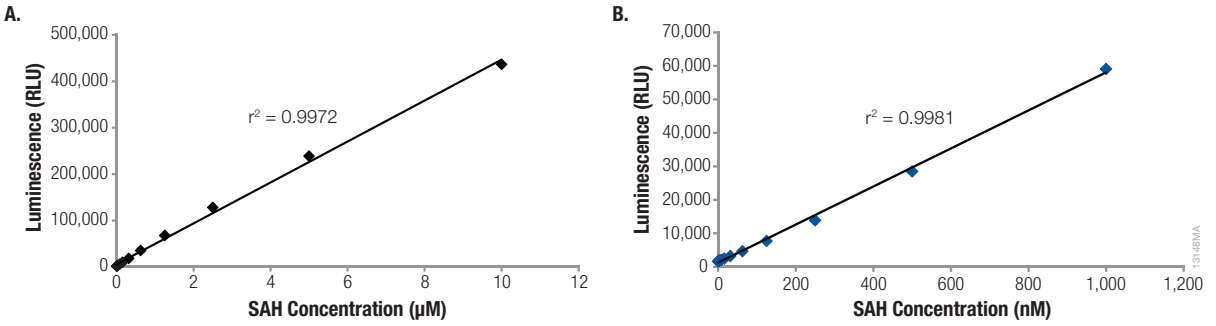

Figure 3. Representative SAH standard curves for the MTase-Glo™ Assay. Four microliters of purified SAH at the indicated concentrations was dispensed into a low-volume 384-well plate. The MTase-Glo™ Assay was performed as described in Section 4.A and luminescence measured using a plate-reading luminometer.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
MTase-Glo™ Methyltransferase Assay	**400 assays**	**V7601**

The system contains sufficient reagents for 400 assays in 96-well plates, or 2,000 assays in low-volume 384-well plates. Includes:

- 300µl SAH, 15µM
- 200µl SAM, 1mM
- 1ml MTase-Glo™ Reagent, 10X
- 10ml MTase-Glo™ Detection Solution

PRODUCT	SIZE	CAT.#
MTase-Glo™ Methyltransferase Assay	**2,000 assays**	**V7602**

The system contains sufficient reagents for 2,000 assays in 96-well plates, or 10,000 assays in low-volume 384-well plates. Includes:

- 300µl SAH, 15µM
- 1ml SAM, 1mM
- 5ml MTase-Glo™ Reagent, 10X
- 50ml MTase-Glo™ Detection Solution

Storage Conditions: Store the MTase-Glo™ Methyltransferase Assay at less than –65°C. Before use, thaw all components completely at room temperature except for the 10X MTase-Glo™ Reagent, which should be thawed on ice. Mix thawed reagents thoroughly before use, but do not vortex. Store the thawed 10X MTase-Glo™ Reagent on ice until ready to use. After the first use, dispense the 10X MTase-Glo Reagent into single-use aliquots and store at less than –65°C. Prepare working dilutions of the MTase-Glo™ Reagent immediately before use, and prepare only enough for each experiment; do not freeze the diluted reagent. After the first use, dispense the thawed MTase-Glo™ Detection Solution into single-use aliquots, and store at –30°C to –10°C. See the product label for expiration date.

3. Before You Begin

3.A Scaling the MTase-Glo™ Methyltransferase Assay

The MTase-Glo™ Methyltransferase Assay can be performed using various plate formats. The protocols in this manual provide recommended volumes for each well of 96-well, half-area 96-well, 384-well, low-volume 384-well and 1,536-well plates.

You can use other volumes, but be sure to scale the reagent volumes proportionally.

3.B. Reaction Buffer and SAH Solution Preparation

Increase or decrease the component volumes provided below to prepare the required volumes of 1X reaction buffer and 1µM SAH Solution for your experiment. The composition of the 4X reaction buffer is provided in Section 5.

1X Reaction Buffer

Component	Volume
NANOpure® water (or water of equivalent purity)	750µl
4X reaction buffer	250µl
Total volume	1ml

1µM SAH Solution

Component	Volume
NANOpure® water (or water of equivalent purity)	136.7µl
15µM SAH	13.3µl
4X reaction buffer	50µl
Total volume	0.2ml

3.C. Preparing Standards for the SAH Standard Curve

To correlate luminescence and SAH concentration, generate a standard curve using serial dilutions of SAH. This protocol describes how to prepare an SAH standard curve ranging from 0µM to 1µM. A representative SAH standard curve is shown in Figure 3. You also may prepare a standard curve ranging from 0µM to 10µM, if desired. Use of an SAH standard curve is optional.

Note: We recommend that you include at least one SAH standard in the range of 0µM to 0.5µM SAH to evaluate luminometer sensitivity. Be sure that your luminometer can measure the net luminescent signal (i.e., the change in luminescence over background) generated from 0.5µM SAH.

Prepare the SAH standards in a separate 96-well plate prior to performing the MTase-Glo™ Methyltransferase Assay, and transfer the standards to wells reserved for the SAH standard curve on the MTase-Glo Assay plate (as described in Section 4). Assay each SAH standard in triplicate. Prepare fresh SAH standards each time you perform the MTase-Glo™ Assay.

1. Add 75µl of 1X reaction buffer to wells A2 through A12 of a 96-well plate.
2. Add 150µl of the 1µM SAH solution prepared in Section 3.B to well A1.
3. Perform a serial twofold dilution by transferring 75µl from well A1 to well A2. Pipet to mix. Transfer 75µl from well A2 to well A3, and pipet to mix. Repeat the transfer for wells A3 through A11, mixing well after each transfer. Discard 75µl from well A11. Do not add SAH solution to well A12. See Figure 4.
4. Store the SAH standards at room temperature until ready to use. Use within 2 hours of preparation.

3.C. Preparing Standards for the SAH Standard Curve (continued)

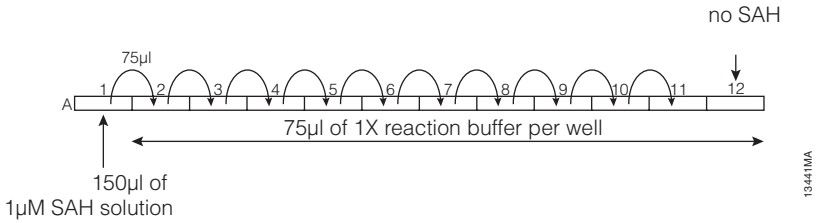

Figure 4. Dilution scheme for preparing SAH standards.

3.D Generating and Using the SAH Standard Curve

The luminescent signal generated by the MTase-Glo™ Methyltransferase Assay is proportional to SAH concentration. To correlate luminescence and SAH concentration, you must generate an SAH standard curve using either raw luminescence (i.e., luminescence without subtracting background) or background-subtracted luminescence. Use the luminescence (in relative light units, RLU) of the SAH standards to generate a standard curve by plotting luminescence (y axis) against SAH concentration (x axis), and generate a linear regression graph using graphing software such as Microsoft Excel®. Use the linear equation to calculate the SAH concentration of a sample. Alternatively, calculate the change in luminescence over background (net RLU) for the SAH standards and samples, and use those background-subtracted luminescence values to generate the standard curve and determine SAH concentration.

For each standard: net RLU = RLU of the standard – RLU of the 0µM SAH standard

For each sample: net RLU = RLU (with enzyme) – RLU (without enzyme)

4. MTase-Glo™ Methyltransferase Assay Protocols

4.A. Determining the Optimal Enzyme Concentration

This protocol allows you to determine the optimal amount of methyltransferase to use in the MTase-Glo™ Assay (often referred to as the EC_{50} value of the enzyme) using a number of appropriate methyltransferase substrates such as peptides, full-length histones and nucleosomes. Alternatively, you can adapt this protocol to quantify enzyme activity. This protocol was developed using low-volume 384-well plates; volumes for other plate formats are also provided. Representative data showing the titration of different classes of methyltransferases are shown in Figure 5.

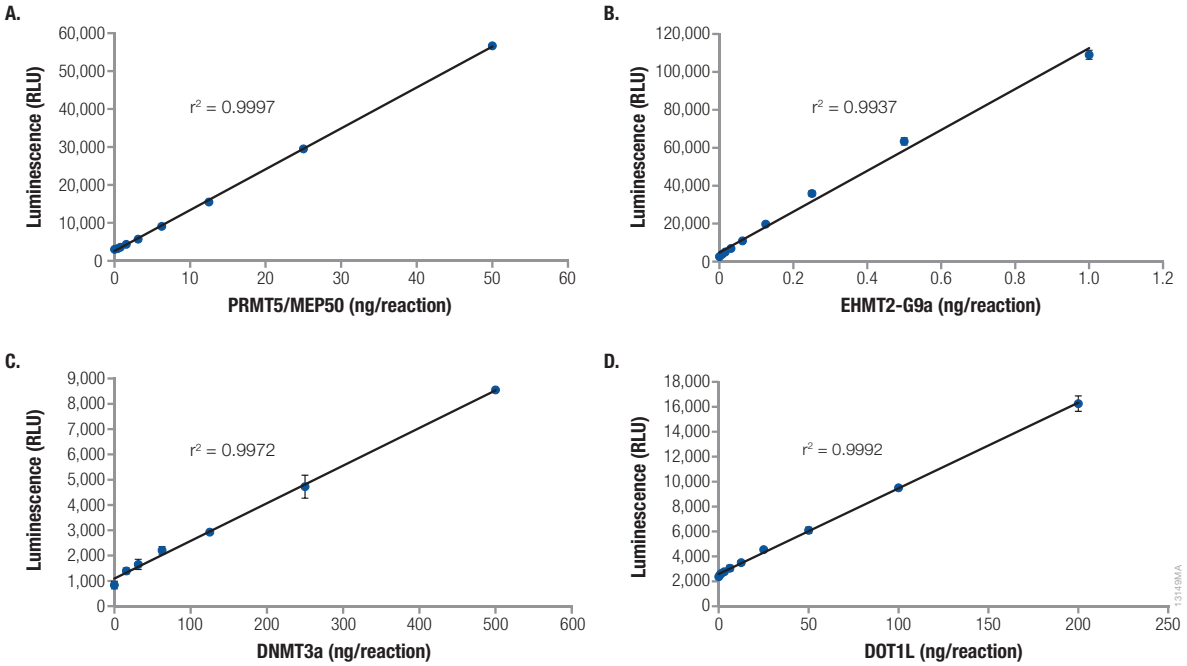

Figure 5. Titration of methyltransferases using the MTase-Glo™ Assay. PRMT5/MEP50 (**Panel A**), EHMT2-G9a (**Panel B**), DNMT3a (**Panel C**) or DOT1/L (**Panel D**) were assayed at the indicated concentration in 1X reaction buffer in a solid white low-volume 384-well plate. The MTase-Glo™ Assay protocol was performed as described in Section 4.A. Each data point represents the average of two replicates; error bars represent the standard deviation. Data analysis was performed using Excel® software. RMT5/MEP50, EHMT2-G9a and DOT1/L were obtained from BPS Bioscience, Inc. (San Diego, CA). DNMT3a was purchased from Reaction Biology Corp (Malvern, PA).

4.A. Determining the Optimal Enzyme Concentration (continued)

Materials to be Supplied by the User

(Solution compositions are provided in Section 5.)

- 4X reaction buffer
- methyltransferase of interest
- methyltransferase substrate of interest other than S-adenosyl-L-methionine (SAM)
- 96-well, 384-well, low-volume 384-well or 1,536-well white assay plate with solid bottom (Do not use black plates or clear plates.)
- 96-well plate for enzyme dilution
- multichannel pipette or automated pipetting station
- table-top centrifuge compatible with the assay plate
- plate shaker
- NANOpure® water or water of equivalent purity
- plate-reading luminometer

Note: If desired, generate an SAH standard curve using serial dilutions of SAH as described in Sections 3.C and 3.D to correlate luminescence and SAH concentration. Be sure to reserve wells on the assay plate or use a separate plate for the SAH standards.

Volume of Reaction Components Required (Per Well)

Reaction Component	Volume for a 96-Well Plate	Volume for a Half-Area 96-Well or 384-Well Plate	Volume for a Low-Volume 384-Well or 1,536-Well Plate
Methyltransferase reaction, including enzyme, substrate and any test compounds	20µl	8µl	4µl
Prepared 5X MTase-Glo™ Reagent	5µl	2µl	1µl
MTase-Glo™ Detection Solution	25µl	10µl	5µl

Preparation

1. Calculate the volume of 1X reaction buffer, 4X reaction buffer, 5X MTase-Glo™ Reagent and MTase-Glo™ Detection Solution required for your experiment. Prepare the 1X reaction buffer as described in Section 3.B. Equilibrate 1X and 4X reaction buffers and MTase-Glo™ Detection Solution to room temperature. The 5X MTase-Glo™ Reagent will be prepared in Step 9.

   SAH Standard (optional): Prepare the SAH standard samples for the SAH standard curve as described in Section 3.C.

2. Prepare the substrate at a 2X concentration with 20µM SAM in 1X reaction buffer as follows:

Component	Volume	Final Concentration
NANOPure® water	to a final volume of 1ml	
4X reaction buffer	250µl	1X
1mM SAM	20µl	20µM
methyltransferase substrate	__µl	2X

   Note: The final concentration of SAM in the methyltransferase reaction will be 10µM (because this is a 2X reaction mix). You may also perform assays at a final SAM concentration of 1µM by preparing the 2X substrate with 2µM SAM (i.e., 2µl of 1mM SAM per 1ml of 2X substrate). To use SAM at a different final concentration, adjust the volume of 1mM SAM accordingly.

3. Prepare 100µl of methyltransferase at the desired concentration in 1X reaction buffer.

4. In a separate 96-well plate, prepare a serial twofold dilution of the methyltransferase enzyme in 1X reaction buffer as follows:

   a. Add 50µl of 1X reaction buffer to wells A2 through A12 of a 96-well plate.

   b. Add 100µl of the enzyme prepared in Step 3 to well A1.

   c. Perform a serial twofold dilution of enzyme by transferring 50µl from well A1 to well A2, and pipet to mix. Transfer 50µl from well A2 to well A3; mix well. Repeat for wells A3 through A11. Discard 50µl from well A11. Do not add enzyme to well A12. See Figure 6.

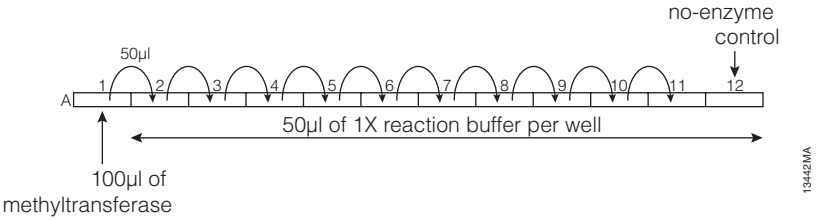

Figure 6. Dilution scheme for the methyltransferase enzyme.

4.A. Determining the Optimal Enzyme Concentration (continued)

5. **SAH Standard (optional):** Transfer the indicated volume of each SAH standard from the 96-well plate prepared in Section 3.C to wells reserved for the SAH standard curve.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
20µl	8µl	4µl

Assay Protocol

6. Add the indicated volume of the 2X substrate prepared in Step 2 to each well of the assay plate.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

7. Start the methyltransferase reaction by transferring the indicated volume of enzyme titration from the 96-well plate prepared in Step 4 to the assay plate. Transfer enzyme from well A1 to all wells in column 1, from well A2 to all wells in column 2, etc.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

Note: Assays in column 12 are the no-enzyme control reactions.

8. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes. Incubate the plate at room temperature (23°C) or at the desired reaction temperature for the desired reaction time.

   Note: While the plate is incubating, thaw the 10X MTase-Glo™ Reagent on ice until ready to use in Step 9.

9. Mix the thawed MTase-Glo™ Reagent thoroughly before use. Prepare the volume of 5X MTase-Glo™ Reagent required in Step 10 by increasing or decreasing the component volumes provided below. Mix gently by inversion; do not vortex. Equilibrate the 5X MTase-Glo™ Reagent to room temperature before use.

Component	Volume
MTase-Glo™ Reagent, 10X	250µl
NANOPure® water (or water of equivalent purity)	250µl
Total volume	500µl

Notes:

1. Prepare the 5X MTase-Glo™ Reagent immediately before use, and prepare only enough for your experiment. Do not freeze.
2. After use, dispense the remaining 10X MTase-Glo™ Reagent into single-use aliquots, and store at less than –65°C.

10. Once the methyltransferase reactions are complete, add the indicated volume of room-temperature 5X MTase-Glo™ Reagent prepared in Step 9 to all wells. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes, and incubate at room temperature for 30 minutes.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
5µl	2µl	1µl

11. Add the indicated volume of room-temperature MTase-Glo™ Detection Solution to all wells. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes, and incubate at room temperature for 30 minutes.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
25µl	10µl	5µl

Note: After use, dispense the remaining MTase-Glo™ Detection Solution into single-use aliquots, and store at –10°C to –30°C.

12. Measure luminescence with a plate-reading luminometer.

Determine the optimal enzyme concentration for your subsequent experiments by plotting luminescence (y axis) against enzyme concentration (x axis) using GraphPad Prism® or similar software.

Note: The optimal enzyme concentration will yield luminescence significantly above background and within the linear range of the assay.

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4.B. Determining K_m Values for a Methyltransferase Substrate

This protocol allows you to determine the K_m value of a potential methyltransferase substrate in the presence of a constant concentration of SAM. Alternatively, you can determine the K_m value of SAM in the presence of a constant concentration of a substrate such as a peptide derived from histone 3 (H3).

When determining K_m, 0.5% TFA is used to stop the reaction before adding the MTase-Glo™ Reagent.

The example protocol provided here uses SAM and H3-derived peptide (1–25) as substrates for the euchromatic histone lysine N-methyltransferase 2 (EHMT2-G9a). With other substrates, optimization of substrate concentration and other assay conditions may be required. Using this protocol, the calculated K_m value for SAM was 0.87µM and for H3-derived peptide (1–25) was 0.85µM; these are similar to the published K_m values (3). Representative results are shown in Figure 7.

Note: If desired, generate an SAH standard curve using serial dilutions of SAH as described in Section 3.C and 3.D to correlate luminescence and SAH concentration. Be sure to reserve wells on the assay plate or a separate plate for the SAH standards.

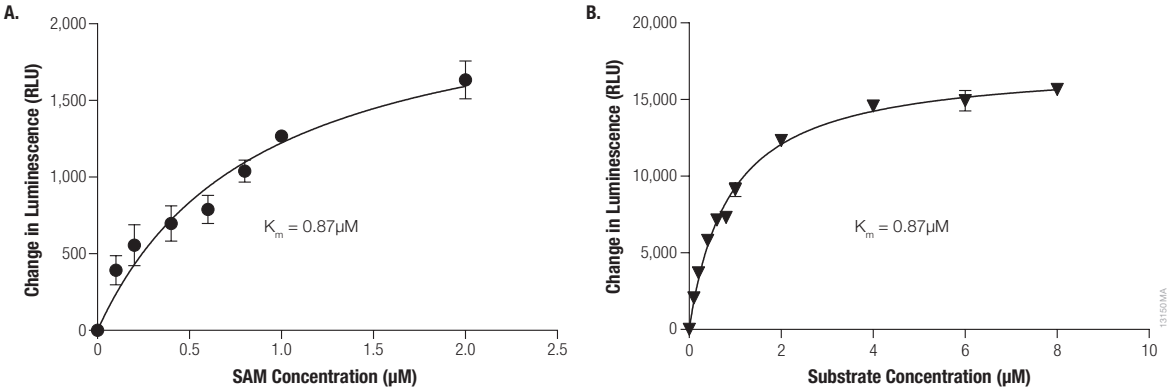


Figure 7. Determining the K_m values of SAM and H3-derived peptide (1–25) for EHMT2-G9a. Panel A. Reactions were assembled with 2ng of EHMT2-G9a enzyme, 20µM H3-derived peptide (1–25) and the indicated concentrations of SAM in wells of a solid white low-volume 384-well plate and incubated for 5 minutes at room temperature (23°C). **Panel B.** Reactions were assembled with 2ng of EHMT2-G9a enzyme, 20µM SAM and the indicated concentrations of H3-derived peptide (1–25) in wells of a solid white low-volume 384-well plate and incubated for 20 minutes at room temperature. For both sets of reactions, the MTase-Glo™ Assay was performed as described in Section 4.B. Each point represents the average of three replicates; error bars represent the standard deviation. Data analysis was performed with GraphPad Prism® software, version 4.02, for Windows® operating systems using a sigmoidal dose-response (variable slope) equation. EHMT2-G9a was obtained from BPS Bioscience, Inc. (San Diego, CA).

Materials to be Supplied by the User
(Solution compositions are provided in Section 5.)

- 4X reaction buffer
- methyltransferase of interest
- methyltransferase substrate of interest such as histone 3-derived peptide (1–25)
- 96-well, 384-well, low-volume 384-well or 1,536-well white assay plate with solid bottom (Do not use black plates or clear plates.)
- 96-well plate for substrate dilution
- multichannel pipette or automated pipetting station
- NANOpure® water or water of equivalent purity
- 0.5% trifluoroacetic acid in NANOPure® water
- table-top centrifuge compatible with the assay plate
- plate shaker
- plate-reading luminometer

Volume of Reaction Components Required (Per Well)

Reaction Component	Volume for a 96-Well Plate	Volume for a Half-Area 96-Well or 384-Well Plate	Volume for a Low-Volume 384-Well or 1,536-Well Plate
Methyltransferase reaction, including enzyme, substrate and any test compounds	20µl	8µl	4µl
0.5% TFA	5µl	2µl	1µl
Prepared 6X MTase-Glo™ Reagent	5µl	2µl	1µl
MTase-Glo™ Detection Solution	30µl	12µl	6µl

Note: In this protocol, the MTase-Glo™ Reagent is used at 6X concentration to compensate for the volume of TFA used in the reaction, and to provide a 1X MTase-Glo™ Reagent concentration in the final reaction.

Preparation

1. Calculate the volume of 1X reaction buffer, 4X reaction buffer, 6X MTase-Glo™ Reagent and MTase-Glo™ Detection Solution required for your experiment. Prepare the 1X reaction buffer as described in Section 3.B. Equilibrate 1X and 4X reaction buffers and MTase-Glo™ Detection Solution to room temperature. The 6X MTase-Glo™ Reagent will be prepared in Step 9.

   SAH Standard (optional): Prepare the SAH standard samples for the SAH standard curve as described in Section 3.C.

4.B. Determining K_m Values for a Methyltransferase Substrate (continued)

2. Prepare the appropriate substrate dilution solution for your experimental design as follows:

   To use a constant concentration of SAM and varying concentrations of the desired substrate such as H3-derived peptide (1–25) in the methyltransferase reaction, prepare substrate dilution solution A with 40µM SAM.

Substrate Dilution Solution A

Component	Volume
NANOPure® water	710µl
4X reaction buffer	250µl
1mM SAM	40µl
Total volume	1.0ml

To use a constant concentration of H3-derived peptide (1–25) and varying concentrations of SAM in the methyltransferase reaction, prepare substrate dilution solution B with 40µM H3-derived peptide (1–25).

Substrate Dilution Solution B

Component	Volume
NANOPure® water	710µl
4X reaction buffer	250µl
1mM H3-derived peptide (1–25)	40µl
Total volume	1.0ml

3. Dilute the enzyme to twice the desired final concentration (2X) in 1X reaction buffer.

4. Prepare the appropriate 2X substrate solution as follows:

   To use a constant concentration of SAM and varying concentrations of H3-derived peptide (1–25), prepare the 2X substrate solution A.

2X Substrate Solution A

Component	Volume	Final Concentration
NANOPure® water	103.5µl	
4X reaction buffer	37.5µl	1X
1mM H3-derived peptide (1–25)	3.0µl	20µM
1mM SAM	6.0µl	40µM
Total volume	150µl	

To use a constant concentration of H3-derived peptide (1–25) and varying concentrations of SAM, prepare the 2X substrate solution B.

2X Substrate Solution B

Component	Volume	Final Concentration
NANOPure® water	103.5µl	
4X reaction buffer	37.5µl	1X
1mM SAM	1.5µl	10µM
1mM H3-derived peptide (1–25)	6.0µl	40µM
Total volume	150µl	

Mix the 2X substrate solution well.

5. In a separate 96-well plate, prepare a serial twofold dilution of the 2X substrate solution prepared in Step 4 as described below. Use substrate dilution buffer A, prepared in Step 2, to dilute the 2X substrate solution A, and use substrate dilution buffer B to dilute the 2X substrate solution B.
   a. Add 50µl of the substrate dilution buffer prepared in Step 2 to wells A2 through A12 of the 96-well plate.
   b. Add 100µl of 2X substrate solution prepared in Step 4 to well A1.
   c. Perform a twofold dilution of the 2X substrate solution by transferring 50µl from well A1 to well A2, and pipet to mix. Transfer 50µl to well A3. Repeat for wells A3 through A11. Discard 50µl from well A11. Do not add substrate to the control reactions in well A12. See Figure 8.

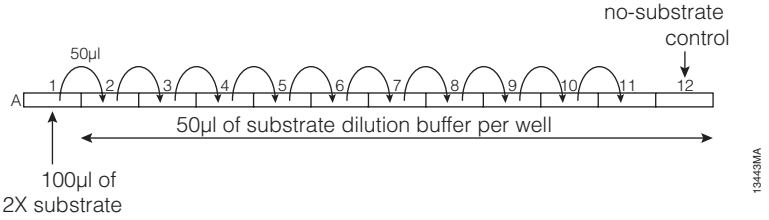


Figure 8. Dilution scheme for the substrate.

6. **SAH Standard (optional):** Transfer the indicated volume of each SAH standard from the 96-well plate prepared in Section 3.C to wells reserved for the SAH standard curve.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
20µl	8µl	4µl

4.B. Determining K_m Values for a Methyltransferase Substrate (continued)

Protocol

7. Add the indicated volume of the 2X substrate solution prepared in Step 5 to each well of the solid white assay plate. Transfer substrate from well A1 to all wells in column 1, from well A2 to all wells in column 2, etc.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

Note: Assays in column 12 are the no-substrate control reactions.

8. Start the methyltransferase reaction by adding the indicated volume of enzyme to each well of the assay plate. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that reagents are at the bottom of the well. Mix well, and incubate at the desired reaction temperature for the desired reaction time.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

Note: During the incubation, thaw the 10X MTase-Glo™ Reagent on ice until ready to use in Step 9.

9. Mix the thawed MTase-Glo™ Reagent thoroughly before use. Prepare the volume of 6X MTase-Glo™ Reagent required in Step 11 by increasing or decreasing the component volumes provided below. Mix gently by inversion; do not vortex. Equilibrate the 6X MTase-Glo™ Reagent to room temperature.

Component	Volume
MTase-Glo Reagent, 10X	312µl
NANOPure® water	208µl
Total volume	520µl

Notes:

1. Prepare the MTase-Glo™ Reagent immediately before use, and prepare only enough for your experiment. Do not freeze the 6X MTase-Glo™ Reagent.
2. Dispense the remaining 10X MTase-Glo™ Reagent into single-use aliquots, and store at less than –65°C.

10. Stop the reaction by adding the indicated volume of 0.5% TFA to each well. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that reagents are at the bottom of the well. Mix well, and incubate for 5 minutes at room temperature.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
5µl	2µl	1µl

11. Add the indicated volume of room-temperature 6X MTase-Glo™ Reagent prepared in Step 9 to all wells. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes, and incubate at room temperature for 30 minutes.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
5µl	2µl	1µl

12. Add the indicated volume of room-temperature MTase-Glo™ Detection Solution to all wells. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes, and incubate at room temperature for 30 minutes.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
30µl	12µl	6µl

Note: After use, dispense the remaining MTase-Glo™ Detection Solution into single-use aliquots, and store at –10°C to –30°C.

13. Measure luminescence using a plate-reading luminometer.

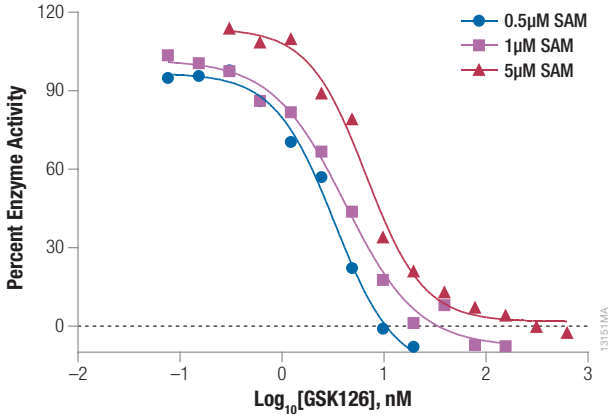
Determine the K_m value using GraphPad Prism® software or similar program.

4.C. Determining IC_{50} Values

This example protocol was developed to determine the IC_{50} value of GSK126 for the histone-lysine N-methyltransferase EZH2 enzyme complex. To use a different combination of enzyme, substrate and test compound, adjust the enzyme, substrate and test compound concentrations as appropriate for your set of experiments.

The MTase-Glo™ Assay is not affected by the presence of up to 2% ethanol or 2% DMSO.

Using this protocol, we determined that the IC_{50} value for GSK126 was 3.28nM at 0.5µM SAM, which is similar to the published IC_{50} value (7,8). Representative results are shown in Figure 9.



SAM (µM)	0.5	1.0	5.0
IC_{50} (nM)	3.28	4.16	6.68

Figure 9. Determining the IC_{50} value of GSK126 for the EZH2 enzyme complex. Reactions were assembled with 0.5µg of EZH2 enzyme complex; 0.5, 1 or 5µM SAM; 10µM full-length histone 3 protein and the indicated concentrations of GSK126 in a solid white low-volume 384-well plate and incubated for 60 minutes at 30°C. The MTase-Glo™ Assay was performed as described in Section 4.C. Each point represents the average of three replicates; the error bars represent the standard deviation. Data analysis was performed using Excel® software. GSK126 was obtained from Xcess Biosciences, Inc. (San Diego, CA).

Materials to be Supplied by the User

(Solution compositions are provided in Section 5.)

- 4X reaction buffer
- EZH2 enzyme complex or methyltransferase of interest
- full-length histone 3 or methyltransferase substrate of interest
- known inhibitor for methyltransferase of interest
- 96-well, 384-well, low-volume 384-well or 1,536-well white assay plate with solid bottom (Do not use black plates or clear plates.)
- 96-well plate for test compound dilution
- multichannel pipette or automated pipetting station
- 200µM GSK126 or a stock solution of the test compound of interest at ten times the highest concentration to be tested
- test compound dilution buffer
- NANOpure® water or water of equivalent purity
- table-top centrifuge compatible with the assay plate
- plate shaker
- plate-reading luminometer

Note: If desired, generate an SAH standard curve using serial dilutions of SAH as described in Section 3.C and 3.D to correlate luminescence and SAH concentration. Be sure to reserve wells on the assay plate or a separate plate for the SAH standards.

Volume of Reaction Components Required (Per Well)

Reaction Component	Volume for a 96-Well Plate	Volume for a Half-Area 96-Well or 384-Well Plate	Volume for a Low-Volume 384-Well or 1,536-Well Plate
Methyltransferase reaction, including enzyme, substrate, MTase-Glo™ Reagent[1] and any test compounds	25µl	10µl	5µl
MTase-Glo™ Detection Solution	25µl	10µl	5µl

[1]The MTase-Glo™ Reagent is included in the methyltransferase reaction mix. See Step 2 for details.

Preparation

1. Calculate the volume of 1X reaction buffer, 4X reaction buffer, 2.5µM SAM and MTase-Glo™ Detection Solution required for your experiment. Prepare the 1X reaction buffer as described in Section 3.B. Equilibrate 1X and 4X reaction buffers and MTase-Glo™ Detection Solution to room temperature.

   SAH Standard (optional): Prepare the SAH standard samples for the SAH standard curve as described in Section 3.C.

4.C. Determining IC_{50} Values (continued)

2. Prepare the EZH2 enzyme complex in 1X reaction buffer with 25µM full-length histone 3 as the substrate.

Component	Volume	Final Concentration
EZH2 enzyme complex	___µl	0.5ng per reaction
full-length histone 3	___µl	25µM
MTase-Glo™ Reagent, 10X	250µl	2.5X
4X reaction buffer	250µl	1X
NANOpure water	to a final volume of 1ml	
Total volume	1ml	

Note: The final concentration of full-length histone 3 in the methyltransferase reaction in Step 9 is 10µM.

3. Prepare the appropriate volume of 2.5µM SAM in 1X reaction buffer as follows:

Component	Volume
1mM SAM	1.25µl
4X reaction buffer	125µl
NANOpure® water	373.75µl
Total volume	500µl

Note: The final concentration of SAM in the methyltransferase reaction in Step 9 is 1µM.

4. Prepare 150µl of 200µM GSK126 stock solution in 1X reaction buffer, and mix well. Perform a serial twofold dilution as follows:
   a. Add 50µl of test compound dilution buffer to wells A2 through A12 of a 96-well plate.
   b. Add 100µl of 200µM GSK126 to well A1.
   c. Transfer 50µl from well A1 to well A2, and pipet to mix. Transfer 50µl to well A3, and mix well. Repeat for wells A3 through A11. Discard 50µl from well A11. Do not add any GSK126 to well A12 for the no-test compound controls. See Figure 10.

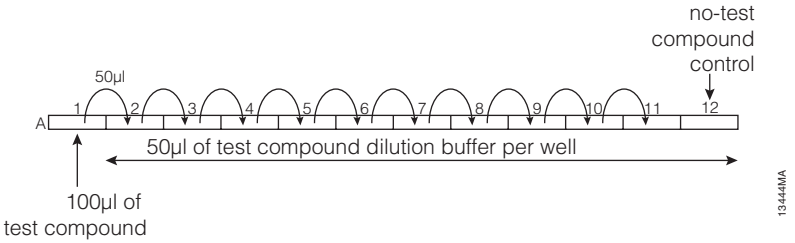


Figure 10. Dilution scheme for the test compound.

5. **SAH Standard (optional):** Transfer the indicated volume of each SAH standard from the 96-well plate prepared in Section 3.C to wells reserved for the SAH standard curve.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
20µl	8µl	4µl

Assay Protocol

6. Add the indicated volume of enzyme prepared in Step 2 to each well of a solid white assay plate.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

7. Transfer the indicated volume of test compound prepared in Step 4 to each well of the assay plate. Transfer test compound from well A1 to all wells in column 1, from well A2 to all wells in column 2, etc.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
5µl	2µl	1µl

8. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that the reagents are at the bottom of the well. Mix well, and incubate for 5–10 minutes at room temperature.

9. Start the methyltransferase reaction by adding the indicated volume of 2.5µM SAM prepared in Step 3 to each well that contains enzyme and test compound. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that the reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes. Incubate the plate at the desired reaction temperature for the desired reaction time.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

10. Add the indicated volume of room-temperature MTase-Glo™ Detection Solution to all wells. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that the reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes, and incubate at room temperature for 60 minutes.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
25µl	10µl	5µl

Note: After use, dispense the remaining MTase-Glo™ Detection Solution into single-use aliquots and store at –10°C –30°C.

11. Measure luminescence using a plate-reading luminometer.

Determine the IC_{50} value of the test compound using GraphPad Prism® software or similar software.

4.D. Determining Z´ Factor

This example protocol was developed to determine the Z´ factor (9) of a reaction system with or without EHMT2-G9a in the presence of 15µM SAM and 15µM H3-derived peptide (1–25) using the MTase-Glo™ Assay. Other methyltransferases and substrates can be used; optimization of assay conditions may be required. Representative data are shown in Figure 11.

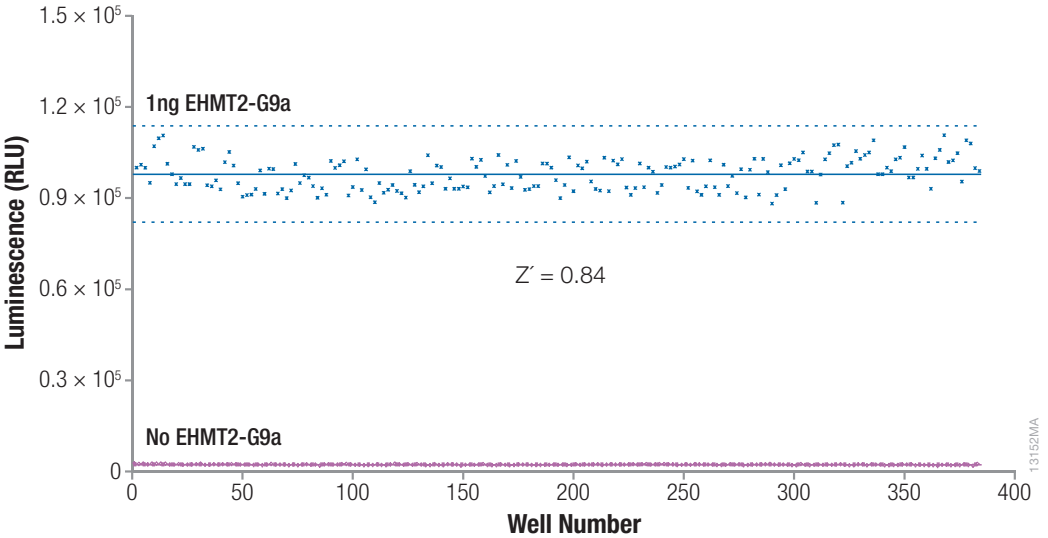


Figure 11. A scatter plot to determine the Z´ factor of the MTase-Glo™ Assay with and without EHMT2-G9a. The MTase-Glo™ Assay was performed in a low-volume 384-well plate as described in Section 4.D. Luminescence was measured using the Infinite® 1000 Pro microplate reader (Tecan). Data analysis was performed using Excel® software. Solid lines indicate the mean, and the dotted lines are ± 3 standard deviations.

Materials to be Supplied by the User

(Solution compositions are provided in Section 5.)

- 4X reaction buffer
- EHMT2-G9a or the methyltransferase of interest
- methyltransferase substrate of interest such as the H3-derived peptide (1–25)
- 96-well, 384-well, low-volume 384-well or 1,536-well white assay plate with solid bottom (Do not use black plates or clear plates.)
- multichannel pipette or automated pipetting station
- NANOpure® water or water of equivalent purity
- table-top centrifuge compatible with the assay plate
- plate shaker
- plate-reading luminometer

Note: If desired, generate an SAH standard curve using serial dilutions of SAH as described in Section 3.C and 3.D to correlate luminescence and SAH concentration. Be sure to reserve wells on the assay plate or a separate plate for the SAH standards.

Volume of Reaction Components Required (Per Well)

Reaction Component	Volume for a 96-Well Plate	Volume for a Half-Area 96-Well or 384-Well Plate	Volume for a Low-Volume 384-Well or 1,536-Well Plate
Methyltransferase reaction, including enzyme, substrate and any test compounds	20µl	8µl	4µl
Prepared 5X MTase-Glo™ Reagent	5µl	2µl	1µl
MTase-Glo™ Detection Solution	25µl	10µl	5µl

Preparation

1. Calculate the volume of 1X reaction buffer, 4X reaction buffer, 5X MTase-Glo™ Reagent and MTase-Glo™ Detection Solution required for your experiment. Prepare the 1X reaction buffer as described in Section 3.B. Equilibrate 1X and 4X reaction buffers and MTase-Glo™ Detection Solution to room temperature. Place the 10X MTase-Glo™ Reagent on ice to thaw. The 5X MTase-Glo™ Reagent will be prepared in Step 5.

   Optional: Prepare the SAH standard samples for the SAH standard curve as described in Section 3.C.

2. Prepare the substrate solution with 15µM SAM and 15µM H3-derived peptide (1–25) in 1X reaction buffer as follows:

Component	Volume
4X reaction buffer	250µl
1mM H3-derived peptide (1–25)	15µl
1mM SAM	15µl
NANOpure® water	720µl
Total volume	1ml

   Mix well.

3. Dilute the EHMT2-G9a in 1X reaction buffer such that you are adding 1ng per reaction in Step 7.

   Note: Use 1ng of EHMT2-G9a per reaction regardless of plate format.

4. **SAH Standard (optional):** Transfer the indicated volume of each SAH standard from the 96-well plate prepared in Section 3.C to wells reserved for the SAH standard curve.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
20µl	8µl	4µl

4.D. Determining Z´ Factor (continued)

5. Mix the thawed MTase-Glo™ Reagent thoroughly before use. Prepare the volume of 5X MTase-Glo™ Reagent required in Step 9 by increasing or decreasing the component volumes provided below. Mix gently by inversion; do not vortex. Equilibrate the 5X MTase-Glo™ Reagent to room temperature before use.

Component	Volume
MTase-Glo™ Reagent, 10X	250µl
NANOPure® water	250µl
Total volume	500µl

Notes:

a. Prepare the 5X MTase-Glo™ Reagent immediately before use, and prepare only enough for your experiment. Do not freeze the 5X MTase-Glo™ Reagent.

b. Dispense the remaining 10X MTase-Glo™ Reagent into single-use aliquots, and store at less than –65°C.

Assay Protocol

6. Prepare the no-EHMT2-G9a reactions by adding the indicated volume of 1X reaction buffer to each well in half of the assay plate. Be sure to reserve wells for the SAH standard curve if desired.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

7. Prepare EHMT2-G9a reactions by adding the indicated volume of EHMT2-G9a in 1X reaction buffer to each well in the other half of the assay plate.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

8. Add the indicated volume of substrate solution prepared in Step 2 to all wells. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
10µl	4µl	2µl

9. Add the indicated volume of room-temperature 5X MTase-Glo™ Reagent prepared in Step 5 to all wells. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that the reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes, and incubate at room temperature for 60 minutes.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
5µl	2µl	1µl

10. Add the indicated volume of room-temperature MTase-Glo™ Detection Solution to all wells. Centrifuge the plate for 1–2 minutes at 1,000rpm using a table-top plate centrifuge to ensure that the reagents are at the bottom of the well. Mix the plate by shaking for 1–2 minutes, and incubate at room temperature for 60 minutes.

96-Well Plate	Half-Area 96-Well or 384-Well plate	Low-Volume 384-Well or 1,536-Well Plate
25µl	10µl	5µl

Note: After use, dispense the remaining MTase-Glo™ Detection Solution into single-use aliquots, and store at –10°C to –30°C.

11. Measure luminescence using a plate-reading luminometer. Determine the Z´ factor using Excel® or similar software.

5. Composition of Buffers and Solutions

4X Reaction Buffer

80mM	Tris buffer, pH 8.0
200mM	NaCl
4mM	EDTA
12mM	$MgCl_2$
0.4mg/ml	BSA
4mM	dithiothreitol (DTT)

Note: When diluted to 1X, the reaction buffer contains 0.1mg/ml of BSA and 1mM DTT to stabilize enzyme activity. If desired, you may use another protein carrier as appropriate for the methyltransferase of interest.

Test Compound Dilution Buffer

20mM	Tris buffer, pH 8.0
50mM	NaCl
1mM	EDTA
3mM	$MgCl_2$
0.1mg/ml	BSA
1mM	dithiothreitol (DTT)
	test compound solvent

Note: The composition of the test compound dilution buffer is nearly identical to that of the 1X reaction buffer; the only difference is the presence of the test compound solvent. To prepare the test compound dilution buffer, add test compound solvent to the 4X reaction buffer such that the final concentration is equal to the solvent concentration of the test compound stock solution in Section 4.C. Dilute the buffer to a final concentration of 1X with NANOpure® water or water of equivalent purity.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and comments
High luminescent signal	One or more reaction components were contaminated with ATP, ADP or other nucleotides. Avoid workspaces and pipettes that are used with ATP- or ADP-containing solutions. Use aerosol-resistant pipette tips. Decontaminate work surfaces by wiping with a detergent solution or ethanol and rinsing with clean water. Rinse pipettes and other labware with distilled water at least three times. For automated dispensing systems, replace any components that have been used to dispense ATP-containing solutions.
	An alternative source of SAM was used to assemble the methyltransferase reactions, and that source was contaminated with SAH. Use only the SAM provided in the MTase-Glo™ Methyltransferase Assay.
	The enzyme was contaminated with ATP or other adenosine nucleotides. Dialyze the enzyme to remove nucleotides. Be sure to store the enzyme in an appropriate buffer at the proper temperature.
	The test compound contained adenosine or its derivative, resulting in increased background luminescence. To detect the presence of adenosine nucleotides, perform two MTase-Glo™ reactions without enzyme or substrate: one with test compound and one without test compound. Compare the luminescence from these reactions. An increase in luminescence in the presence of test compound indicates contamination by adenosine or its derivatives. **Note:** During a screen of the 1,280 compound LOPAC library (Sigma), we observed less than 0.5% false hits, and only two compounds increased luminescence.

Symptoms	Causes and comments
Low luminescent signal	Incorrect assay plate was used. Use only solid white plates. Do not use black or clear plates.
	Improper storage of the MTase-Glo™ reagents. Prepare the MTase-Glo™ Reagent immediately before use, and prepare only enough for your experiment; do not freeze the prepared MTase-Glo™ Reagent. Gently mix the MTase-Glo™ Reagent and MTase-Glo™ Detection Solution before use. Store unused 10X MTase-Glo™ Reagent at less than –65°C in single-use aliquots. Store unused MTase-Glo™ Detection Solution at –10°C to –30°C in single-use aliquots. Avoid repeated freeze-thaw cycles of both reagents.
	Substrate was not added to methyltransferase reaction. Be sure to add the substrate to reactions. Include SAH standards as positive control reactions.
	The test compound inhibited a component of the MTase-Glo™ Detection Solution (e.g, the ATP-generating enzyme or luciferase). To identify inhibitors, add 10µM adenosine to two tubes, and add test compound to one tube but not the other tube. Perform the MTase-Glo™ Assay. Compare the luminescence from these reactions. A decrease in luminescence in the presence of the test compound indicates inhibition of the MTase-Glo™ Assay.
	To identify luciferase inhibitors, add 1µM ATP to two tubes, and add test compound to one tube but not the other tube. Perform the MTase-Glo™ Assay but omit the MTase-Glo™ Reagent. Compare the luminescence from these reactions. Low light output in the presence of the test compound indicates luciferase inhibition. **Note:** During a screen of the 1,280 compound LOPAC library (Sigma), we observed less than 0.2% false hits, and only two of the compounds inhibited luciferase.
	The reaction was inhibited by the test compound solvent. Minimize the solvent concentration, or use a different solvent to dissolve the test compound. Perform control reactions that contain solvent but no test compound to test the effect of the solvent on assay performance. The MTase-Glo™ Assay is not affected by the presence of up to 2% ethanol or 2% DMSO.
	Luminometer was not sensitive enough. Be sure to use a luminometer that can measure the net luminescent signal (i.e., the change in luminescence over background) generated from 0.5µM SAH. The SAH standards prepared in Section 3.C can be used to gauge luminometer sensitivity.

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7. References

1. Luo, M. (2012) Current chemical biology approaches to interrogate protein methyltransferases. *ACS Chem. Biol.* **7**, 443–63.
2. Lu, Q. *et al.* (2012) Perspectives on the discovery of small-molecule modulators for epigenetic processes. *J. Biomol. Screen.* **17**, 555–71.
3. Carr, S.M. and La Thangue, N.B. (2011) Cell cycle control by a methylation-phosphorylation switch. *Cell Cycle* **10**, 733–4.
4. Roadmap Epigenomics Consortium (2015) Integrative analysis of 111 reference human epigenomes. *Nature* **518**, 317–30.
5. Okano, M. *et al.* (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* **99**, 247–57.
6. Horiuchi, K. Y. *et al.* (2013) Assay development for histone methyltransferases. *Assay Drug Dev. Technol.* **11**, 227–36.
7. Ott, H.M. *et al.* (2014) A687V EZH2 is a driver of histone H3 lysine 27 (H3K27) hypertrimethylation. *Mol. Cancer Ther*. **13**, 3062–73.
8. McCabe, M.T. *et al.* (2012) EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature* **492**, 108–12.
9. Zhang, J., Chung, T.D. and Oldenburg, K.R. (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* **4**, 67–73.

8. Related Products

Product	Size	Cat.#
AMP-Glo™ Assay*	1,000 assays	V5011
ADP-Glo™ Kinase Assay*	1,000 assays	V9101
UDP-Glo™ Glycosyltransferase Assay*	200 assays	V6961
Kinase-Glo® Luminescent Kinase Assay*	10ml	V6711
GTPase-Glo™ Luminescent Assay System	1,000 assays	V7681
	10,000 assays	V7682
Succinate-Glo™ JmjC Demethylase/Hydroxylase Assay*	1,000 assays	V7990

*Additional sizes available.

Luminometers

Product	Size	Cat.#
GloMax® Discover System	each	GM3000
GloMax® Explorer System Fully Loaded Model	each	GM3500
GloMax® Explorer System with Luminescence and Fluorescence	each	GM3510
GloMax® 96 Microplate Luminometer	each	E6501
GloMax® 96 Microplate Luminometer w/Single Injector	each	E6511
GloMax® 96 Microplate Luminometer w/Dual Injectors	each	E6521

9. Summary of Changes

The following changes were made to the 3/24 revision of this document:

1. Corrected the figure legend of Figure 9. to change 0.5ng to 0.5µg.
2. Updated font and cover image.
3. Made minor text edits.

[a]U.S. Pat. Nos. 7,741,067, 8,361,739 and 8,603,767, Japanese Pat. No. 4485470 and other patents pending.

[b]U.S. Pat. Nos. 6,602,677, 7,241,584, 8,030,017 and 8,822,170, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.