Description

The G9a Chemiluminescent Assay Kit is designed to measure G9a activity for screening and profiling applications. This kit comes in a convenient format, with a plate precoated with histone H3 peptide substrate, recombinant human G9a enzyme (amino acids 785-1210), high specific antibody against the methylated lysine residue of Histone H3, secondary HRP-labeled antibody, S-adenosylmethionine and Methyltransferase Assay Buffer for 100 enzyme reactions.

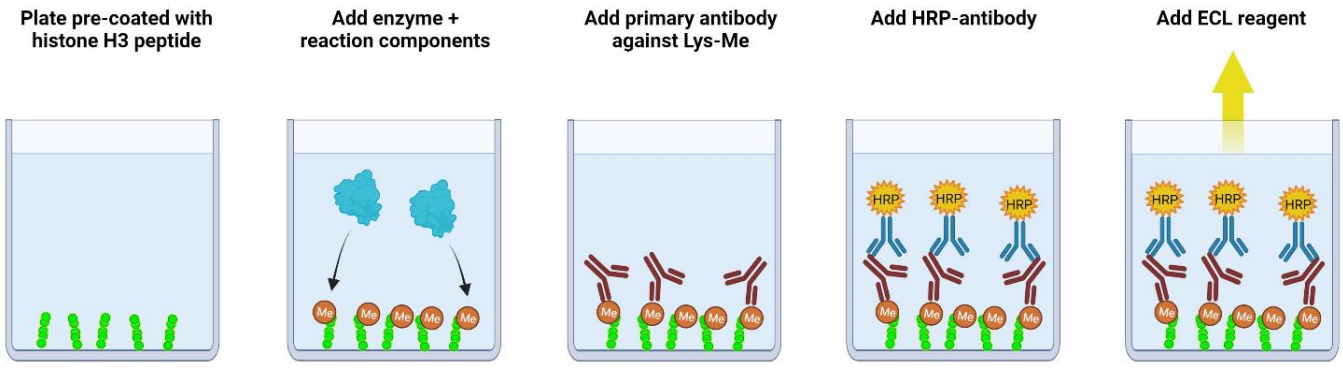

Figure 1: G9a Chemiluminescent Assay Kit workflow diagram. First, S-adenosylmethionine is incubated with the assay buffer, G9a enzyme, and the inhibitor of choice for one hour in a histone-precoated plate. This is followed by the addition of a primary antibody against Lys(Me). Addition of a chemiluminescent HRP substrate provides a luminescence signal, which directly correlates with the activity of G9a.

Background

G9a, also known as euchromatic histone-lysine N-methyltransferase 2 or EHMT2, belongs to the histone methyltransferase enzyme family. It is involved in mono- and di-methylation of histone H3, at lysine 9 and lysine 27, which control epigenetic regulation of pluripotency during embryogenesis, by depositing H3K9me2 in the promoters of genes like OCT4 (octamer-binding transcription factor 4). It is also involved in neuropathic pain, by regulating a large number of genes in the dorsal root ganglia. G9a has been implicated in cancer cell growth, having a key role in self-renewal of cancer stem cells (CSCs). The presence of CSC populations leads to cancer resurfacing after treatment and they tend to be resistant to typical cancer treatments. The use of inhibitors targeting G9a can result in toxicity, such as in the case of UNC0642 or BIX-01294. The development of newer context-specific inhibitors will provide new therapeutic avenues in cancer therapy.

Applications

Study enzyme kinetics and screening small molecule inhibitors for drug discovery and high-throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
51001	G9a (EHMT2), GST-Tag (Sf9-derived)*	8 µg	-80°C
52120	20 µM S-adenosylmethionine	250 µl	-80°C
52140E	Primary Antibody 5	12.5 µl	-80°C
52130H	Secondary HRP-Labeled Antibody 1	10 µl	-80°C
52160	4x HMT Assay Buffer 1	3 ml	-20°C
52100	Blocking Buffer 4	50 ml	+4°C
	0.5 M DTT	200 µl	-20°C
	HRP Chemiluminescence Substrate (2 components)	6 ml each	+4°C
	96-well module plate (pre-coated with Histone Substrate)**	1 plate	+4°C

**The concentration of the protein is lot-specific and will be indicated on the tube.*
***Custom coated plates (example, with a lower concentration of Histone Substrate) are available upon request.*

Materials Required but Not Supplied

- TBST Buffer (1x TBS, containing 0.05% Tween-20), pH 8
- Luminometer or plate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, “No Substrate Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

Step 1

1. Rehydrate the microwells by adding 150 µl of TBST buffer, pH 8 to every well.

2. Incubate 15 minutes at Room Temperature (RT).

3. Tap the strip onto clean paper towels to remove liquid.

4. Thaw **S-adenosylmethionine** on ice. Briefly spin the tube to recover the full content.

5. Add 125 µl of **0.5 M DTT** to **4x HMT Assay Buffer 1**.

6. Prepare a **Master Mix** (25 µl/well): N wells × (7.5 µl 4x HMT Assay Buffer 1 + 2.5 µl 20 µM S-adenosylmethionine + 15 µl distilled water).

   Note: Decreasing of S-adenosylmethionine concentration can allow higher sensitivity to the inhibitors.

7. Add 25 µl of Master Mix to all wells, except the "No Substrate Control" wells.

8. Prepare a **Deficient Master Mix** for the "No Substrate Control" wells (25 µl/well): N wells × (7.5 µl 4x HMT Assay Buffer 1 + 17.5 µl distilled water).

9. Add 25 µl of Deficient Master Mix to the "No Substrate Control" wells.

10. Dilute 4-fold the 4x HMT Assay Buffer 1 (containing DTT) with distilled water. This makes 1x HMT Assay Buffer 1.

11. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

    11.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x HMT Assay Buffer 1, 10-fold more concentrated than the desired final concentrations.

    For the positive and negative controls, use 1x HMT Assay Buffer 1 (Diluent Solution).

    OR

    11.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x HMT Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

    Using 1x HMT Assay Buffer 1 in 10% DMSO, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations to keep the concentration of DMSO constant.

    For positive and negative controls, prepare 10% DMSO in 1x HMT Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO in the assay should not exceed 1%.

12. Add 5 µl of Test Inhibitor to each well labeled as "Test Inhibitor".
13. Add 5 µl of Diluent Solution to the "Positive Control", "No Substrate Control" and "Blank" wells.
14. Thaw **G9a** enzyme on ice. Briefly spin the tube containing enzyme to recover the full content.
15. Dilute G9a enzyme to 2-4 ng/µl (20 µl/well) with 1x HMT Assay Buffer 1.
16. Add 20 µl of diluted G9a to all wells, except the "Blank" wells.
17. Add 20 µl of 1x HMT Assay Buffer 1 to the "Blank" wells.
18. Incubate for 1 hour at RT with gentle agitation.
19. Wash three times with 200 µl TBST Buffer.
20. Tap the plate onto clean paper towel to remove the liquid.
21. Add 100 µl of Blocking Buffer 4 to every well.
22. Incubate for 10 minutes at RT with gentle agitation.
23. Tap the plate onto clean paper towel to remove the liquid.

	Blank	No Substrate Control	Positive Control	Test Inhibitor
Master Mix	25 µl	-	25 µl	25 µl
Deficient Master Mix	-	25 µl	-	-
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
1x HMT Assay Buffer 1	20 µl	-	-	-
Diluted G9a (2-4 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2

1. Dilute 800-fold the **Primary Antibody 5** with Blocking Buffer 4.
2. Add 100 µl of diluted Primary Antibody 5 to each well.
3. Incubate for 1 hour at RT with gentle agitation.
4. Wash three times with 200 µl of TBST Buffer.
5. Tap the plate onto clean paper towel to remove the liquid.

6. Add 100 µl of Blocking Buffer 4 to every well.
7. Incubate for 10 minutes at RT with gentle agitation.
8. Tap the plate onto clean paper towel to remove the liquid.

Step 3

1. Dilute 1,000-fold the **Secondary HRP-Labeled Antibody 1** in Blocking Buffer 4.
2. Add 100 µl of diluted Secondary HRP-Labeled Antibody 1 to each well.
3. Incubate for 30 minutes at RT with gentle agitation.
4. Wash three times with 200 µl of TBST Buffer.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 µl of Blocking Buffer 4 to every well.
7. Incubate for 10 minutes at RT with gentle agitation.
8. Tap the plate onto clean paper towel to remove the liquid.
9. Just before use, mix 1 volume of **HRP Chemiluminescence Substrate A** and 1 volume of **HRP Chemiluminescence Substrate B** (100 µl of mix/well).
10. Add 100 µl to each well.
11. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
12. The "Blank" value should be subtracted from all other values.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry. To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Validation Data

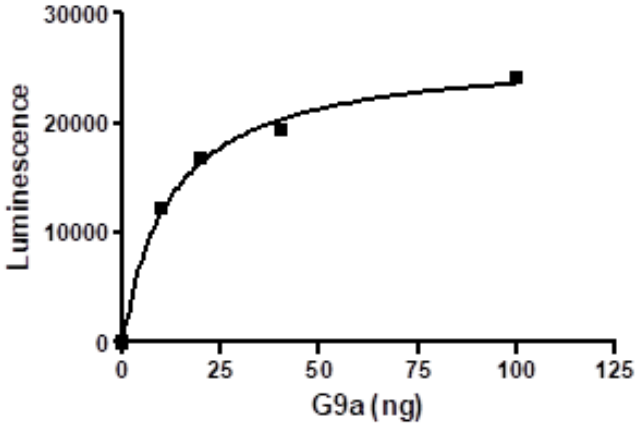

Figure 2: G9a activity.
G9a activity was measured in the presence of increasing amounts of G9a. Luminescence was measured using a Bio-Tek microplate reader.

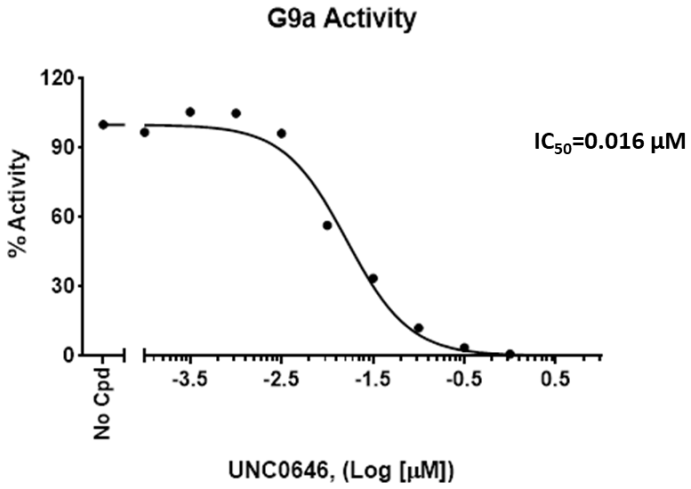

Figure 2: Inhibition of G9a activity by UNC0646.
G9a activity was measured in the presence of increasing concentrations of UNC0646. Results are expressed as percent of control (G9a activity measured in the absence of inhibitor, set at 100%). Luminescence was measured using a Bio-Tek microplate reader.

Data shown is representative, for lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Dillon S. C., *et al.*, 2005 *Genome Biology* 6:227.
Haebe J., *et al*., 2021 *Oncogenesis* 10: 76.

Related Products

Products	*Catalog #*	*Size*
G9a (EHMT2), GST-Tag (E. coli-derived) Recombinant	51000	50 µg
G9a Homogeneous Assay Kit	52051	384 reactions
Histone H3(K9) Universal Methyltransferase Assay Kit	52072	96 reactions
SUV39H1 Chemiluminescent Assay Kit	52006L	96 reactions
SUV39H2 Activity Assay Kit	52008L	96 reactions