



6042 Cornerstone Court West, Ste. B  
San Diego CA 92121  
Tel: 1.858.202.1401  
Fax: 1.858.481.8694  
Email: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

## Data Sheet **G9a Chemiluminescent Assay Kit** Catalog # 52001L

**DESCRIPTION:** The *G9a Chemiluminescent Assay Kit* is designed to measure G9a activity for screening and profiling applications. The *G9a Chemiluminescent Assay Kit* comes in a convenient format, with wells precoated with histone H3 peptide substrate, the antibody against methylated lysine residue of Histone H3, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified G9a enzyme for 100 enzyme reactions. The key to the *G9a Direct Activity Assay Kit* is a highly specific antibody that recognizes methylated K9 residue of Histone H3. With this kit, only three simple steps are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme for one hour. Next, primary antibody is added. Finally, the strips are treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

### COMPONENTS:

Catalog #	Component	Amount	Storage	
51001	G9a human enzyme	8 µg	-80°C	<b>(Avoid freeze/thaw cycles!)</b>
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	
	HRP chemiluminescent substrate (2 components)	6 ml each	+4°C	
	96-well plate precoated with histone substrate***	1 plate	+4°C	

\*) *Decreasing of S-adenosylmethionine concentration will make the assay more sensitive to the inhibitors.*

\*\*) *Add 125 µl of 0.5 M DTT before use.*

\*\*\*) *Custom coated plates with a lower concentration of histone H3 peptide substrate are available upon request.*

### MATERIALS REQUIRED BUT NOT SUPPLIED:

TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween20)

Luminometer or fluorescent microplate reader capable of reading chemiluminescence

Adjustable micropipettor and sterile tips

Rotating or rocker platform

0.5 M DTT

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**APPLICATIONS:** Great for studying enzyme kinetics and HTS applications.

**CONTRAINDICATIONS:** DMSO >1%, strong acids or bases, ionic detergents, high salt

**STABILITY:** One year from date of receipt when stored as directed.

**REFERENCE:** Dillon SC, Zhang X, Trievel RC, Cheng X. *Genome Biology* 2005; **6**:227.

**ASSAY PROTOCOL:**

**All samples and controls should be tested in duplicate.**

**Step 1:**

- 1) Rehydrate the microwells by adding 150  $\mu$ l of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the strip onto clean paper towels to remove liquid.
- 2) Thaw **S-adenosylmethionine** on ice. Upon first thaw, briefly spin tube containing **S-adenosylmethionine** to recover full content of the tube. Aliquot **S-adenosylmethionine** into single use aliquots. Store remaining **S-adenosylmethionine** in aliquots at  $-80^{\circ}\text{C}$  immediately. *Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*
- 3) Add 125  $\mu$ l of 0.5 M DTT to 4x HMT assay buffer 1. Prepare the master mixture: N wells  $\times$  (7.5  $\mu$ l **4x HMT assay buffer 1** + 2.5  $\mu$ l 20  $\mu$ M **S-adenosylmethionine** + 15  $\mu$ l  $\text{H}_2\text{O}$ )
- 4) Dilute **4x HMT assay buffer 1** to **1x HMT assay buffer 1** in  $\text{dH}_2\text{O}$ .
- 5) Thaw **G9a enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **G9a enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at  $-80^{\circ}\text{C}$  immediately. *Note: G9a enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 6) Dilute **G9a enzyme** in **1x HMT assay buffer 1** to 2 – 4 ng/ $\mu$ l (40-80 ng/20  $\mu$ l). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
- 7) Add 25  $\mu$ l of master mixture to each well designated for the "Positive Control", "Test Inhibitor," and "Blank". For the "Substrate Control", add 7.5  $\mu$ l **4x HMT assay buffer 1** + 17.5  $\mu$ l  $\text{H}_2\text{O}$ .

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	Blank	Substrate Control	Positive Control	Test Inhibitor
4x HMT assay buffer 1	7.5 µl	7.5 µl	7.5 µl	7.5 µl
20 µM S-adenosylmethionine	2.5 µl	–	2.5 µl	2.5 µl
H <sub>2</sub> O	15 µl	17.5 µl	15 µl	15 µl
Test Inhibitor/Activator	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1x HMT assay buffer 1	20 µl	–	–	–
G9a (2 – 4 ng/µl)	–	20 µl	20 µl	20 µl
<b>Total</b>	<b>50 µl</b>	<b>50 µl</b>	<b>50 µl</b>	<b>50 µl</b>

- 8) Add 5 µl of inhibitor solution of each well designated “Test Inhibitor”. For the “Positive Control”, “Substrate Control” and “Blank”, add 5 µl of the same solution without inhibitor (inhibitor buffer).
- 9) Add 20 µl of **1x HMT buffer 1** to the well designated “Blank”.
- 10) Initiate reaction by adding 20 µl of diluted **G9a** prepared as described above. Incubate at room temperature for 1 hour.
- 11) Wash the strip three times with 200 µl TBST buffer. Blot dry onto clean paper towels.
- 12) Add 100 µl of **Blocking buffer 4** to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

## Step 2:

- 1) Dilute **Primary antibody 5** 800-fold with **Blocking buffer 4**.
- 2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.
- 3) Wash strip three times with 200 µl TBST buffer and incubate in **Blocking buffer 4** as in steps 1-10 and 1-11.

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### Step 3:

- 1) Dilute **Secondary HRP-labeled antibody 1** 1,000-fold with **Blocking buffer 4**.
- 2) Add 100  $\mu$ l per well. Incubate for 30 minutes at room temperature with slow shaking.
- 3) Wash strip three times with TBST buffer and incubate in **Blocking buffer 4** as in steps 1-10 and 1-11.
- 4) Just before use, mix on ice 50  $\mu$ l **HRP chemiluminescent substrate A** and 50  $\mu$ l **HRP chemiluminescent substrate B** and add 100  $\mu$ l per well. Discard any unused chemiluminescent reagent after use.
- 5) Immediately read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

### Reading Chemiluminescence:

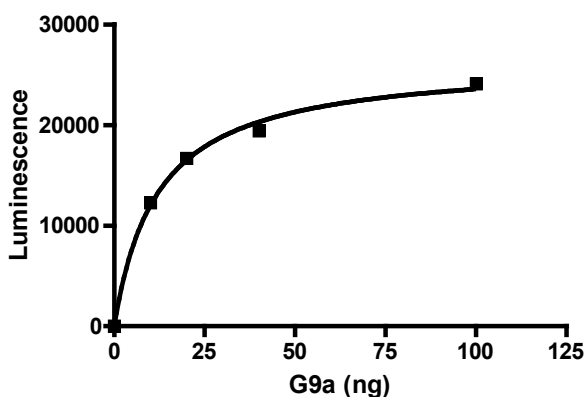
Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

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).

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**Example of Assay Results:**



G9a enzyme activity, measured using the *G9a Chemiluminescent Assay Kit*, BPS Bioscience Catalog #52001L. Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com).*

**RELATED PRODUCTS**

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
G9a enzyme ( <i>E. coli</i> )	#51000	50 µg
G9a enzyme (Sf9 cells)	#51001	20 µg
G9a Homogeneous Assay Kit	#52051	384 reactions
H3(K9) Universal Methyltransferase Assay Kit	#52072	96 reactions
SUV39H1 (82-end) enzyme	#51070	50 µg
SUV39H1 (full length) enzyme	#51071	5 µg
SUV39H2 enzyme	#51080	50 µg
SUV39H1 Chemiluminescent Assay Kit	#52045	96 reactions
SUV39H2 Chemiluminescent Assay Kit	#52008	96 reactions

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### TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	G9A enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (G9A, BPS Bioscience #51000). Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for antibody incubation. Avoid freeze/thaw cycles of antibody.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap strip lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of enzyme (G9A, BPS Bioscience #51000) to create a standard curve.

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