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Data Sheet
EHMT1 (GLP) Chemiluminescent Assay Kit
Catalog # 53007
Size: 96 reactions

DESCRIPTION: The *EHMT1 (GLP) Chemiluminescent Assay Kit* is designed to measure EHMT1 (also known as G9a-like protein) activity for screening and profiling applications. The *EHMT1 (GLP) Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well plate precoated with histone H3 peptide substrate, primary antibody against methylated lysine residue of Histone H3, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified EHMT1 for 96 enzyme reactions. The key to the *EHMT1 (GLP) Chemiluminescent Assay Kit* is a highly specific antibody that recognizes methylated K9 residue of H3. With this kit, only three simple steps on a microtiter plate 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 plate is treated with an HRP-labeled secondary antibody followed by addition of the ELISA ECL substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51020	EHMT1 (GLP)	4 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
52140E	Primary antibody 5	12.5 µl	-80°C	
52130H	Secondary HRP-labeled antibody 1	10 µl	-80°C	
52120	20 µM S-adenosylmethionine	250 µl	-80°C	
52160	4x HMT assay buffer 1*	3 ml	-20°C	
79556	Blocking buffer 1	50 ml	+4°C	
79670	ELISA ECL substrate (2 components)	6 ml each	Room Temp.	
	96-well plate precoated with histone substrate	1 plate	+4°C	

*Add 125 µl of 0.5M DTT.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween-20)
Luminometer or microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

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

REFERENCE: Huang, J., *et al., J Biol. Chem.* 2010 Mar 26; **285(13)**: 9636-41.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 200 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the plate 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 contents of the tube. Aliquot **S-adenosylmethionine** into single use aliquots and store at -80°C. *Note: **S-adenosylmethionine** is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

3) Add 125 µl of 0.5M DTT. Prepare the master mixture: N wells × (7.5 µl **4x HMT assay buffer 1** + 2.5 µl **20 µM S-adenosylmethionine** + 15 µl H_2O). Add 25 µl of master mixture to all wells labeled "Positive Control", "Test Sample", and "Blank". For wells labeled "Substrate Control", add 7.5 µl **4x HMT assay buffer 1** + 17.5 µl H_2O.

	Blank	Substrate Control	Positive Control	Test Sample
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_2O	15 µl	17.5 µl	15 µl	15 µl
Test Inhibitor	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1x HMT assay buffer 1	20 µl	–	–	–
Diluted EHMT1/GLP (2 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

4) Add 5 µl of inhibitor solution of each well designated "Test Inhibitor".

5) For the "Positive Control", "Substrate Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer).

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6) Thaw **EHMT1 (GLP) enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **EHMT1 (GLP) enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **EHMT1 (GLP)** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Dilute **EHMT1 (GLP) enzyme** in **1x HMT assay buffer 1** at 2 ng/µl (40 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use. *Note: Diluted enzyme may not be stable. Dilute the enzyme immediately before use.*

8) Add 20 µl of **1x HMT buffer 1** to the wells designated “Blank”.

9) Initiate reaction by adding 20 µl of diluted **EHMT1 (GLP)** enzyme to the wells designated “Positive Control”, "Substrate Control", and "Test Sample ". Incubate at room temperature for 1 hour.

10) Remove the supernatant from the wells and wash the plate three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

11) Add 100 µl of **Blocking buffer** 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**.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Remove the supernatant from the wells and wash the plate three times with 200 µl TBST buffer and incubate in **Blocking buffer** as in steps 1-10 and 1-11.

Step 3:

1) Dilute “**Secondary HRP-labeled antibody 1**” 1,000-fold with **Blocking buffer**.

2) Add 100 µl per well. Incubate for 30 min at room temperature with slow shaking.

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3) Remove the supernatant from the wells and wash the plate three times with 200 µl TBST buffer and incubate in **Blocking buffer** as in steps 1-10 and 1-11.

4) Just before use, mix on ice 50 µl **ELISA ECL substrate A** and 50 µl **ELISA ECL substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

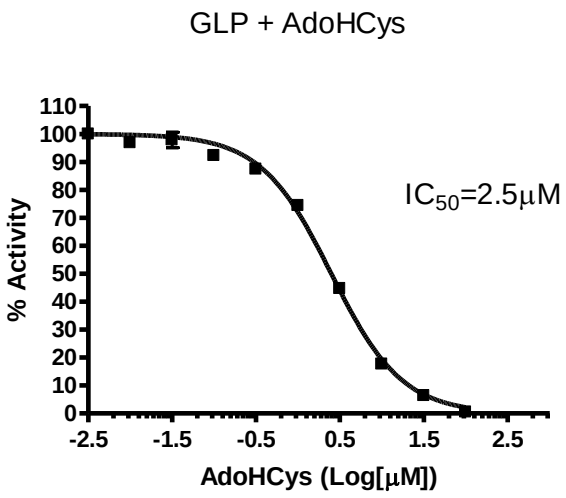
5) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

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).

Example of Assay Results:



EHMT1 (GLP) enzyme activity, measured using the *EHMT1 (GLP) Chemiluminescent Assay Kit*, BPS Bioscience Catalog #53007. Luminescence was measured using a Bio-Tek fluorescent

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microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS

Product Name	Catalog #	Size
EHMT1 (GLP)) enzyme	51020	50 µg
G9a (EHMT1) enzyme (*E. coli*)	51000	50 µg
G9a (EHMT1) enzyme (Sf9 cells)	51001	20 µg
SUV39H1 (82-end) enzyme	51070	50 µg
SUV39H1(full length) enzyme	51071	5 µg
SUV39H2 enzyme	51080	50 µg
G9a Homogeneous Assay Kit	52051	384 reactions
G9a Chemiluminescent Assay Kit	52001L	96 reactions
SUV39H1 Chemiluminescent Assay Kit	52045	96 reactions
SUV39H2 Chemiluminescent Assay Kit	52008	96 reactions
H3(K9) Universal Methyltransferase Assay Kit	52072	96 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is same as "blank" value.	EHMT1 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh EHMT1 (GLP), BPS Bioscience #51020. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection. See "Reading Chemiluminescence" section above.
	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 plate 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 EHMT1 (GLP), BPS Bioscience #51020 to create a standard curve.