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Data Sheet

Histone H3(K9) Universal Methyltransferase Assay Kit

Catalog #52072

DESCRIPTION: The *Histone H3(K9) Universal Methyltransferase Assay Kit* is designed for the detection of histone H3(K9) methyltransferase (HMT) activity in screening and profiling applications. Histone H3(K9) methyltransferases are enzymes that catalyze the transfer of a methyl group from the cofactor S-adenosylmethionine to lysine 9 residue of histone H3. The *Histone H3(K9) Universal Methyltransferase Assay Kit* comes in a convenient format, with a 96-well plate precoated with histone H3 peptide substrate, antibody against methylated lysine 9 residue of Histone H3, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified G9a, SUV39H1 and SUV39H2 enzymes for 100 enzyme reactions. The key to the *Histone H3(K9) Universal Methyltransferase Assay Kit* is a highly specific antibody that recognizes methylated K9 residue of Histone 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 plates 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	
51000	G9a human recombinant enzyme	4 µg	-80°C	***Avoid freeze/ thaw cycles!***
51070	SUV39H1 human recombinant enzyme	4 µg	-80°C	
51080	SUV39H2 human recombinant enzyme	2 µg	-80°C	
	100 µM S-adenosylmethionine	250 µl	-80°C	
52140A	Primary antibody 1	100 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	50 ml	+4°C	
	HRP chemiluminescent substrate A (translucent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
	8-well strip plate precoated with histone substrate	1	+4°C	

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

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

STABILITY: Up to 1 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 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 strip plate onto clean paper towels to remove liquid.

2) Thaw **S-adenosylmethionine** on ice. Upon first thaw, briefly spin the 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) Dilute 100 µM **S-adenosylmethionine** 4-fold with water to make a 25 µM solution. Dilute only the amount of **S-adenosylmethionine** required for the assay. Discard any unused diluted **S-adenosylmethionine** after use.

4) Prepare the master mixture: N wells x (7.5 µl **4x HMT Assay Buffer 1** + 5 µl diluted (25 µM) **S-adenosylmethionine** + 12.5 µl H_2O). Add 25 µl of master mixture to all wells labeled “Positive Control”, “Test Inhibitor”, and “Blank”. For wells labeled “Substrate control”, add 7.5 µl **4x HMT Assay Buffer 1** + 17.5 µl water.

5) Add 5 µl of inhibitor solution of each well designated “Test Inhibitor”.

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

7) Thaw **G9a**, **SUV39H1**, and **SUV39H2** enzymes on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **G9a**, **SUV39H1**, and **SUV39H2** enzymes into single use aliquots. Store remaining undiluted enzymes

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in aliquots at -80°C. *Note: All 3 enzymes are very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzymes.*

	Blank	Substrate Control	Positive Control	Test Inhibitor
4x HMT assay buffer 1	7.5 µl	7.5 µl	7.5 µl	7.5 µl
25 µM S-adenosylmethionine	5 µl	–	5 µl	5 µl
H_2O	12.5 µl	17.5 µl	12.5 µl	12.5 µl
Test Inhibitor	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1x HMT assay buffer 1	20 µl	–	–	–
G9a (5 ng/µl) or SUV39H1 (5 ng/µl) or SUV39H2 (2.5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

8) Dilute **G9a** enzyme in **1x HMT assay buffer 1** to 5 ng/µl (100 ng/20 µl). Dilute **SUV39H1** in **1x HMT Assay Buffer 1** to 5 ng/µl (100 ng/20 µl). Dilute **SUV39H2** in **1x HMT Assay Buffer 1** to 2.5 ng/µl (50 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

9) Add 20 µl of **1x HMT Assay Buffer 1** to the well designated “Blank”.

10) Initiate reaction by adding 20 µl of diluted enzyme to the wells designated “Positive Control”, "Substrate Control", and "Test Inhibitor". Incubate at room temperature for 1 hour.

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

12) 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 1**” 100-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 strip three times with 200 µl TBST buffer and incubate in **Blocking Buffer** as described in steps 1-11 and 1-12.

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Step 3:

1) Dilute "**Secondary HRP-labeled antibody 1"** 1,000-fold with **Blocking Buffer**.

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

3) Remove the supernatant from the wells and wash the strip three times with 200 µl TBST buffer and incubate in **Blocking Buffer** as described in steps 1-11 and 1-12.

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

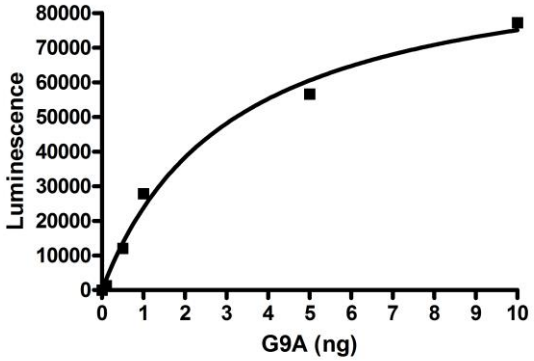
5) Immediately read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

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. Do not use a filter when measuring light emission. Optimal settings will vary depending on the particular plate reader. Typical integration time is 1 second, delay after plate movement is 100 msec. Typical settings for the Synergy 2 Bio-Tek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on luminescence of a control assay without enzyme (typically we set this value as 100).

Example of Assay Results:



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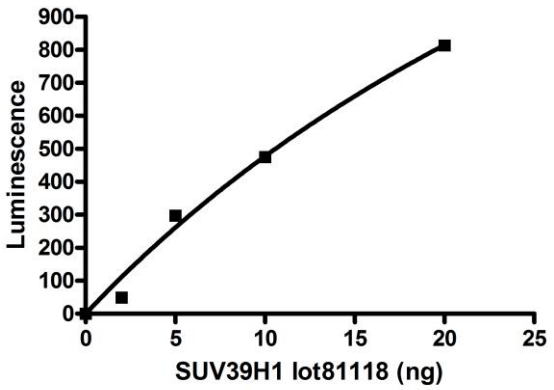

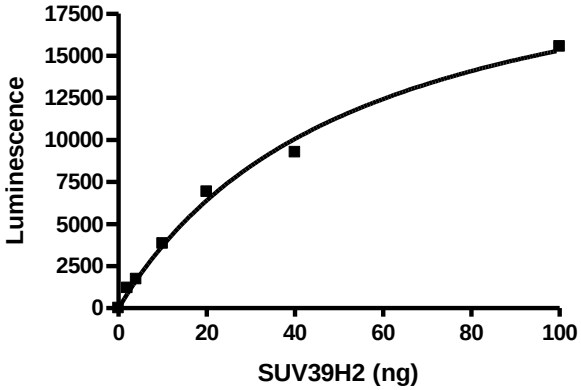

G9a, SUV39H1, and SUV39H2 enzyme activity, measured using the Histone H3(K9) Universal Methyltransferase Assay Kit, BPS Bioscience #52072. Luminescence was measured using a Bio-Tek fluorescent microplate reader.

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

RELATED PRODUCTS

G9a (expressed in *E. coli*)	#51000	50 µg
G9a (expressed in Sf9 cells)	#51001	20 µg
SUV39H1 (82-end)	#51070	50 µg
SUV39H1 (full length)	#51071	5 µg
SUV39H2	#51080	50 µg
G9a Chemiluminescent Assay Kit	#52001L	96 reactions
SUV39H1 Chemiluminescent Assay Kit	#52006L	96 reactions
SUV39H2 Chemiluminescent Assay Kit	#52008	96 reactions
G9a Assay Kit, Homogeneous	#52051	384 reaction
Chaetocin	#27221	1 mg
UNC0638	#27222	5 mg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	Methyltransferase enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzymes (G9a, BPS Bioscience #51000; SUV39H1, #51070; SUV39H2, #51080). Store enzymes 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	Record light signals at 5 second intervals. 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.
Chemiluminescence signal is erratic or varies widely	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/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; SUV39H1, #51070; SUV39H2, #51080) to create a standard curve.