6044 Cornerstone Court West, Ste. E
San Diego CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

SUV39H1 Chemiluminescent Assay Kit

Catalog # 52006L
Size: 96 reactions

DESCRIPTION: The *SUV39H1 Chemiluminescent Assay Kit* is designed to measure SUV39H1 activity for screening and profiling applications. The *SUV39H1 Chemiluminescent Assay Kit* comes in a convenient format, with 8-well strips 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 SUV39H1 enzyme for 96 enzyme reactions. The key to the *SUV39H1 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. Next, primary antibody is added. Finally, the plate is 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	
51070	SUV39H1 human enzyme	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
52120	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	
79670	ELISA ECL substrate A (transparent bottle)	6 ml	+4°C	
79670	ELISA ECL substrate B (brown bottle)	6 ml	+4°C	
	96-well plate precoated with histone substrate	1 plate	+4°C	

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

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

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

REFERENCE: Dillon, S.C., *et al. 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 (1**×** 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 × (7.5 µl **4× 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 Sample” and “Blank”. For wells labeled “Substrate control”, add 7.5 µl **4X HMT assay buffer 1** + 17.5 µl water.

	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	–
1× HMT assay buffer 1	20 µl	–	–	–
SUV39H1 (5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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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 **SUV39H1 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **SUV39H1** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **SUV39H1 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

8) Dilute **SUV39H1** enzyme in **1× HMT assay buffer 1** to 5 ng/µl (100 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 enzyme immediately before use.*

9) Add 20 µl of **1× HMT buffer 1** to the well designated “Blank”.

10) Initiate reaction by adding 20 µl of diluted **SUV39H1** enzyme to the wells designated “Positive Control”, "Substrate Control", and "Test Sample". 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.

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.

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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.

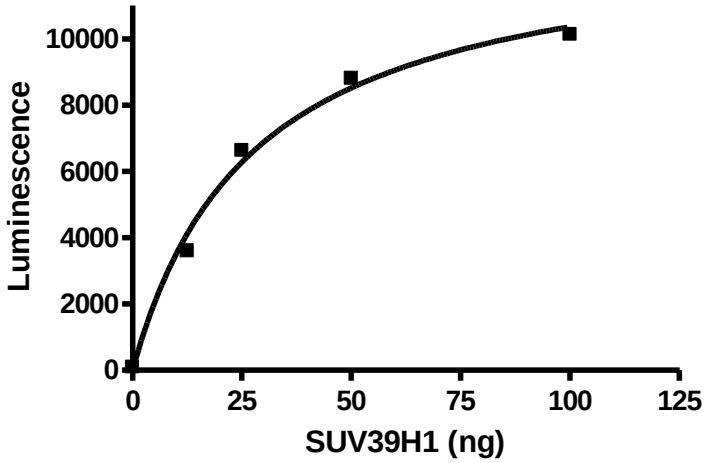
5) Immediately read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

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:



SUV39H1 enzyme activity, measured using the *SUV39H1 Chemiluminescent Assay Kit*, BPS Bioscience Catalog #52006L. 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.*

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RELATED PRODUCTS

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

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	SUV39H1 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (SUV39H1, BPS Bioscience #51070). 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. Refer to the section "Reading Chemiluminescence".
	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 the 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 enzyme (SUV39H1, BPS Bioscience #51070) to create a standard curve.

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