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Data Sheet

SUV39H2 Activity Assay Kit

Catalog # 52008L

DESCRIPTION: The *SUV39H2 Direct Activity Assay Kit* is designed to measure SUV39H2 activity for profiling and screening applications, using purified SUV39H2. The *SUV39H2 Direct Activity Assay Kit* comes in a convenient format, with a 96-well plate 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 SUV39H2 enzyme for 100 enzyme reactions. The key to the *SUV39H2 Activity 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 is added to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Cat. #	Component	Amount	Storage	
51080	SUV39H2	4 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	
	HRP chemiluminescent substrate (2 components)	6 ml each	+4°C	
	96-well plate precoated with histone substrate	1 plate	+4°C	

*Add 125 µl of 0.5 M DTT before use.

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
0.5 M DTT
Rotating or rocker platform
Paper towels

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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: 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 (1 x 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 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) Add 125 µl of 0.5 M DTT to 4x HMT Assay Buffer 1. Prepare the master mix: 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 Sample”, and “Blank”. For wells labeled “Substrate Control”, add 7.5 µl **4x HMT Assay Buffer 1** + 7.5 µl H_2O.

5) Add 5 µl of test sample solution to each well labeled “Test Sample”.

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

7) Thaw **SUV39H2** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **SUV39H2** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **SUV39H2** enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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8) Dilute **SUV39H2** enzyme in **1x HMT Assay Buffer** to 2 ng/µl (40 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

9) Add 20 µl **1x HMT assay buffer** to the well designated "Blank".

10) Initiate reaction by adding 20 µl of dilute **SUV39H2** to the wells designated "Positive Control", "Test Sample", and "Substrate Control". Incubate at room temperature for 1 hour.

11) Remove the supernatant from the wells and wash the strip three times with 200 µl of 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.

	Positive Control	**Test Sample**	**Substrate Control**	**Blank**
SUV39H2 (2 ng/µl)	20 µl	20 µl	20 µl	–
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
Test Inhibitor/Activator	–	X µl	–	–
Test Sample Buffer	5 µl	–	5 µl	5 µl
1x HMT Assay Buffer 1	–	–	–	20 µl
H_2O	12.5 µl	12.5 – X µl	17.5 µl	12.5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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.**

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

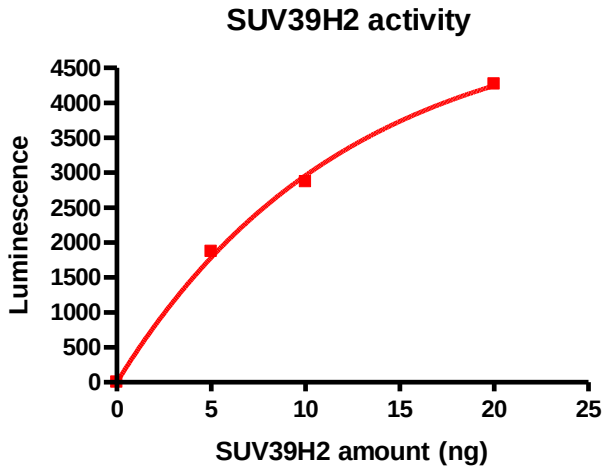
3) Remove the supernatant from the wells and wash the strip three times with 200 µl TBST buffer and incubate in **Blocking Buffer** as described in steps 1-11 and 1-12.
4) Just before use, mix on ice 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

5) Immediately read sample in a luminometer or micro-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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SUV39H2 enzyme activity, measured using the SUV39H2 Assay Kit, BPS Bioscience #52008L. 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

SUV39H1 (82-end) enzyme	#51070	50 µg
SUV39H1(full length) enzyme	#51071	5 µg
SUV39H2 enzyme	#51080	50 µg
SUV4-20H1 enzyme	#51090	50 µg
SUV4-20H2 enzyme	#51060	50 µg
SUV39H1 Chemiluminescent Assay Kit	#52045	96 reactions
EZH2 Chemiluminescent Assay Kit	#52009L	96 reactions
G9a Chemiluminescent Assay Kit	#52001L	96 reactions
SET7/9 Chemiluminescent Assay Kit	#52003L	96 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	SUV39H2 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (SUV39H2, BPS Bioscience #51080). 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 antibodies.
	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 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 (SUV39H2, BPS Bioscience #51080) to create a standard curve.

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