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## **Data Sheet**

### **SUV39H2 NEW Direct Activity Assay Kit**

**Catalog # 52008**

**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, HRP-labeled antibody against methylated lysine residue of Histone H3, S-adenosylmethionine, methyltransferase assay buffer, and purified SUV39H2 enzymes for 100 enzyme reactions. The key to the *SUV39H2 Direct 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, HRP-labeled antibody is added. Finally, HRP substrate is added to produce chemiluminescence that can then be measured using a chemiluminescence reader.

#### **COMPONENTS:**

	Cat. #		Amount	Storage
<b>(Avoid freeze/thaw cycles!)</b>	51080	SUV39H2	4 µg	-80 °C
	52120	400 µM S-adenosylmethionine	250 µl	-80 °C
	...	HRP-labeled antibody 1	50 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
		Black plate precoated with histone substrate	1	+4 °C

#### **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  
Paper towels

**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:** 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  $\mu$ l 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 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.*

3) Dilute SUV39H2 enzyme in 1X HMT assay buffer 1 to 1–2 ng/ $\mu$ l. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

4) Using master mixes as much as possible, add the following reagents to the microwells of the substrate-coated black plate, in duplicate:

	Positive Control	Test Sample	Substrate Control	Blank
SUV39H2 (1–2 ng/ $\mu$ l)	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	–
4x HMT assay buffer 1	12.5 $\mu$ l	12.5 $\mu$ l	12.5 $\mu$ l	12.5 $\mu$ l
400 $\mu$ M S-adenosylmethionine	2.5 $\mu$ l	2.5 $\mu$ l	–	2.5 $\mu$ l
Test Inhibitor/Activator	–	X $\mu$ l	–	–
H <sub>2</sub> O	15 $\mu$ l	15 - X $\mu$ l	17.5 $\mu$ l	35 $\mu$ l
<b>Total</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>

5) Incubate at room temperature for 1 hour.

6) Wash the plate three times with TBST buffer. Blot dry onto clean paper towels.

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- 7) Add 100  $\mu$ l of Blocking buffer to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

**Step 2:**

- 1) Dilute "HRP-labeled antibody 1" 200-fold with Blocking buffer.
- 2) Add 100  $\mu$ l per well. Incubate 1 hour at room temperature with slow shaking.
- 3) Wash plate three times with TBST buffer and incubate in Blocking buffer as in steps 1-6 and 1-7.
- 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. Incubate 5-10 min.

**Step 3:**

Read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

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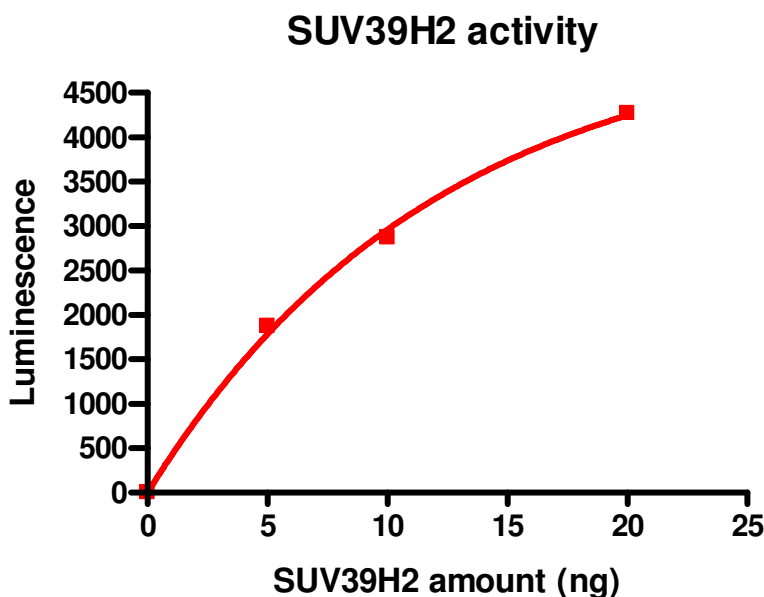
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**Example of Assay Results:**



SUV39H2 enzyme activity, measured using the SUV39H2 Assay Kit, BPS Bioscience #52008. 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**

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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