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Data Sheet
SETD2 *Chemiluminescent Assay Kit*
Catalog # 52060

DESCRIPTION: The *SETD2 Chemiluminescent Assay Kit* is designed to measure SETD2 activity for screening and profiling applications. The *SETD2 Chemiluminescent Assay Kit* comes in a convenient strip format, with a 96-well plate precoated with histone H3 peptide substrate, the antibody against methylated lysine36 residue of Histone H3, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified SETD2 enzyme for 100 enzyme reactions. The key to the *SETD2 Chemiluminescent Assay Kit* is a highly specific antibody that recognizes methylated K36 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 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	
53019	SETD2 human enzyme	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
52120	400 µM S-adenosylmethionine	250 µl	-80°C	
52140L	Primary antibody 12	100 µl	-80°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
52160	4x HMT assay buffer 1*	3 ml	-20°C	
79556	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 0.5M DTT before use.

MATERIALS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween20)
Luminometer or microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips

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: 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 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 content of the tube. Aliquot S-adenosylmethionine into single use aliquots. Store remaining S-adenosylmethionine in aliquots at -80°C immediately. *Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

3) Add 125 µl of 0.5M DTT before use. Prepare the master mixture: N wells × (7.5 µl **4× HMT assay buffer 1** + 2.5 µl **400 µM S-adenosylmethionine** + 15 µl **H_2O**)

4) Thaw **SETD2 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **SETD2 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **SETD2 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Dilute **SETD2 enzyme** in 1× HMT assay buffer 1 at 5 ng/µl (100 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

   Add 25 µl of master mixture to each well designated for the “Positive Control”, “Test Inhibitor”, and “Blank”. For the “Substrate Control”, add 7.5 µl **4× HMT assay buffer 1** + 17.5 µl **H_2O**

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	Blank	Substrate Control	Positive Control	Test Inhibitor
4× HMT assay buffer 1	7.5 µl	7.5 µl	7.5 µl	7.5 µl
400 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/Activator	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1× HMT assay buffer 1	20 µl	–	–	–
SETD2 (5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

6) Add 5 µl of inhibitor solution of each well designated “Test Inhibitor”. For the “Positive Control”, “Substrate Control” and “Blank”, add 5 µl of the same solution without inhibitor (inhibitor buffer)

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

8) Initiate reaction by adding 20 µl of diluted SETD2 prepared as described above. Incubate at room temperature for 1 hour.

9) Wash the plate three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

10) 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 12”** 100-fold with Blocking buffer.

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

3) Wash plate three times with 200 µl TBST buffer and incubate in Blocking buffer as in steps 1-9 and 1-10.

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Step 3:

1) Dilute "**Secondary HRP-labeled antibody 2**" 1,000-fold with Blocking buffer.

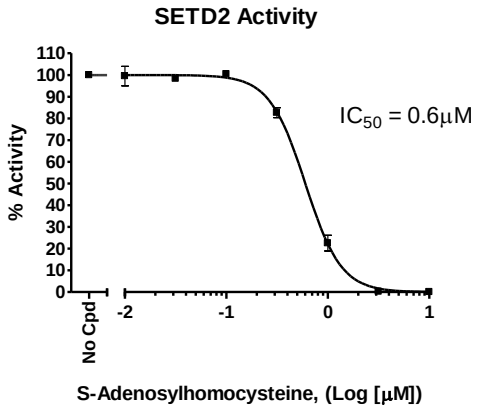
2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash plate three times with TBST buffer and incubate in Blocking buffer as in steps 1-9 and 1-10.

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.

Step 4: Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Example of Assay Results:



SETD2 enzyme activity, measured using the *SETD2 Chemiluminescent Assay Kit*, BPS Bioscience Catalog #52060. 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 support@bpsbioscience.com.*

RELATED PRODUCTS

SET7/9	#51010	100 µg
SET8	#51008	50 µg
SUV39H1 Assay Kit	#52006L	96 reactions
SUV39H2 Assay Kit	#52007L	96 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	SETD2 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (SETD2, BPS Bioscience #53019). 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	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.
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 to create a standard curve.