Description

The SetDB1 Chemiluminescent Assay Kit is designed to measure SetDB1 activity for screening and profiling applications. The SetDB1 Chemiluminescent Assay Kit comes in a convenient 96-well format, precoated with histone H3 peptide substrate, an antibody against the methylated lysine 9 residue of Histone H3, a secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified SetDB1 enzyme (amino acids 510-1290) for 96 enzyme reactions. The advantage of the SetDB1 Chemiluminescent Assay Kit is a highly specific antibody that recognizes the methylated K9 residue of Histone H3. With this kit, only three simple steps are required. First, S-adenosylmethionine is incubated with a sample containing the assay buffer and the methyltransferase enzyme. Next, the primary antibody is added. Finally, the wells are treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.

Background

SetDB1 (Histone-lysine N-methyltransferase SETDB1) is a methyltransferase that reversibly catalyzes the di- and trimethylation of histone 3 (H3) resulting in inhibition of gene transcription within the euchromatic regions of chromosomes. Studies linking the activity of SetDB1 and H3 methylation can elucidate the mechanisms of tumorigenesis in various types of cancers.

Application(s)

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Materials Provided

Catalog #	Name	Amount	Storage
51055	SetDB1, His-GST-Tags*	2 µg	-80°C
52120	20 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 4	50 ml	4°C
	0.5 M DTT	200 µl	-20°C
	HRP Chemiluminescent Substrate A (transparent bottle)	6 ml	4°C
	HRP Chemiluminescent Substrate B (brown bottle)	6 ml	4°C
	96-well plate precoated with histone substrate		4°C

**The concentration of the protein is lot-specific and will be indicated on the tube.*

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Materials Required but Not Supplied

Name	Ordering Information
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween-20)	
Luminometer	
Orbital Shaker	

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly

Contraindications

- This assay is compatible with DMSO >1%.
- This assay is incompatible with strong acids or bases, ionic detergents and high salt.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control", "Substrate Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

Step 1

1. Rehydrate the microwells of the pre-coated plate by adding 150 µl of TBST (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well.

2. Incubate the plate for 15 minutes at Room Temperature (RT).

3. Remove the liquid by inverting the pre-coated plate and tapping onto clean paper towels.

4. Thaw **20 µM S-adenosylmethionine** on ice. Briefly spin the tube containing S-adenosylmethionine to recover its full content.

5. Add 125 µl of **0.5 M DTT** to 3 ml of **4x HMT Assay Buffer 1** before use.

6. Prepare 1x HMT Assay Buffer by diluting 4-fold the 4x HMT Assay Buffer 1 (with DTT) with distilled water.

7. Prepare a Master Mix (25 µl/well): N wells × (7.5 µl of 4X HMT Assay Buffer 1 (with DTT) + 2.5 µl of 20 µM S-adenosylmethionine + 15 µl of distilled water).

8. Add 25 µl of Master Mix to the "Positive Control", "Test Inhibitor" and "Blank" wells.

9. Add 25 µl of 1X HMT Assay Buffer 1 to the “Substrate Control” wells.

10. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

    10.1 If the Test inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using 1x HMT Assay Buffer.

    For the positive and negative controls, use 1x HMT Assay Buffer (Diluent Solution).

    OR

    10.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO then dilute the inhibitor 10-fold in 1x HMT Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

    Using HMT Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

    For positive and negative controls, prepare 10% DMSO in HMT Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

    Note: The final concentration of DMSO should not exceed 1%.

11. Add 5 µl of Test inhibitor dilutions to the “Test Inhibitor” wells.

12. Add 5 µl of the Diluent Solution to the “Positive Control", “Substrate Control” and “Blank” wells.

13. Thaw **SetDB1** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

14. Dilute SetDB1 enzyme to 1 ng/µl (20 µl/well) in 1x HMT Assay Buffer.

    Note: Diluted enzyme may not be stable. Dilute the enzyme immediately before use. The initial concentration of the enzyme is lot-specific and is indicated on the tube containing the enzyme. Discard any unused diluted enzyme after use.

15. Add 20 µl of 1x HMT Buffer 1 to the “Blank” wells.

16. Initiate the reaction by adding 20 µl of the diluted SetDB1 enzyme to the wells designated “Positive Control”, "Substrate Control", and "Test Inhibitor".

17. Incubate at RT for 1 hour.

18. Remove the supernatant from the wells and wash three times with 200 µl of TBST buffer.

19. Blot dry onto clean paper towels.
20. Add 100 µl of **Blocking Buffer 4** to every well.
21. Agitate gently on an orbital platform for 10 minutes.
22. Remove the buffer by inverting the plate and blot dry onto clean paper towels.

Step 2

1. Dilute 100-fold the Primary Antibody 1 with Blocking Buffer 4.
2. Add 100 µl of diluted Primary Antibody 1 per well.
3. Incubate 1 hour at RT with gentle agitation.
4. Wash three times with 200 µl of TBST buffer.
5. Blot dry onto clean paper towels.
6. Add 100 µl of Blocking Buffer 4 to every well and incubate on an orbital shaker for 10 minutes.
7. Remove the Blocking Buffer by inverting the plate and blot dry onto clean paper towels.

Step 3

1. Dilute 1,000-fold the **Secondary HRP-labeled Antibody 1** with Blocking Buffer 4.
2. Add 100 µl of diluted Secondary HRP-labeled Antibody 1 per well.
3. Incubate for 30 minutes at RT with gentle agitation.
4. Wash three times with 200 µl TBST buffer.
5. Add 100 µl of Blocking Buffer 4 to every well and incubate on an orbital shaker for 10 minutes.
6. Remove Blocking Buffer 4 by inverting the plate and blot dry onto clean paper towels.
7. On ice, mix 50 µl of HRP Chemiluminescent Substrate A and 50 µl of HRP Chemiluminescent Substrate B.
8. Add **immediately** 100 µl of the mix to each well.

   Note: Prepare the chemiluminescent substrate just before use. Discard any unused chemiluminescent reagent after use.

9. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.
10. The “Blank” value should be subtracted from all readings.

	Blank	Positive Control	Test Inhibitor	Substrate Control
Master Mix	25 µl	25 µl	25 µl	-
Test Inhibitor	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	5 µl
1x HMT Assay Buffer 1	20 µl	-	-	25 µl
Diluted SetDB1 (1 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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

Validation Data

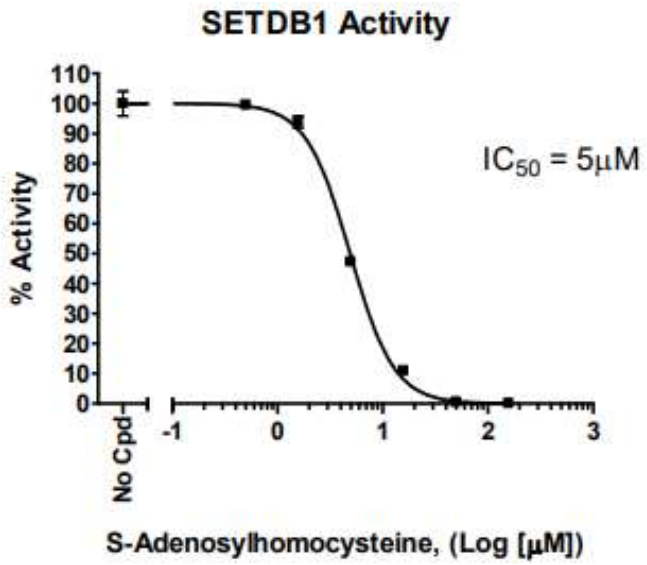

Figure 1: Inhibition of SetDB1 enzymatic activity by SAH.
SetDB1 activity was measured in the presence of increasing concentrations of inhibitor S-Adenosylhomocysteine (SAH). Luminescence was measured using a Bio-Tek fluorescent microplate reader.

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

References

Torrano J., *et al.,* 2019 *Clinical Epigenetics*. 11(1):43.
Dillon S.C., *et al.*, 2005 *Genome Biol.* 6(8):227.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
SetDB1 Chemiluminescent Assay Kit	51056-2	384 reactions
DNMT1 Chemiluminescent Assay Kit	52050L	96 reactions