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 384-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 for 384 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 molecular inhibitors for drug discovery and high throughput (HTS) applications.

Materials Provided

Catalog #	Name	Amount	Storage	
51055	SetDB1 Human Enzyme*	4 µg	-80°C	
52120	20 µM S-adenosylmethionine	2 x 250 µl	-80°C	
52140A	Primary Antibody 1	2 x 100 µl	-80°C	***Avoid multiple freeze/ thaw cycles***
52130H	Secondary HRP-labeled Antibody 1	2 x 10 µl	-80°C	
52160	4x HMT assay buffer 1**	2 x 3 ml	-20°C	
52100	Blocking Buffer 4	2 x 50 ml	4°C	
	HRP chemiluminescent substrate A (transparent bottle)	2 x 6 ml	4°C	
	HRP chemiluminescent substrate B (brown bottle)	2 x 6 ml	4°C	
	384-well plate precoated with histone substrate		4°C	

**The concentration of the protein is lot-specific and will be indicated on the tube.*
***Add 125 µl of 0.5M DTT to 3 mL of 4x HMT assay buffer before use.*

Materials Required but Not Supplied

Name	Catalog #
DTT (0.5 mM in water, prepared fresh)	
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween-20)	
Luminometer	
Rotating or rocker platform	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when materials are stored as directed. ***Avoid multiple freeze/thaw cycles***.

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

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

Assay Protocol

- All samples and controls should be performed in duplicates
- The assay should include a “Blank” and a “Positive control”

Step 1

1. Rehydrate the microwells of the pre-coated plate by adding 100 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.

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

4. Thaw S-adenosylmethionine on ice. Briefly spin the tube containing S-adenosylmethionine to recover its full contents.

5. Calculate the amount of S-adenosylmethionine required for your assay. Aliquot the remaining S-adenosylmethionine into single-use aliquots and store the at -80°C immediately.

Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

6. Add 125 µl of freshly prepared 0.5 M DTT to 3 ml vial of 4x HMT assay buffer 1 before use.

7. Prepare 1x HMT assay buffer by diluting 4x HMT assay buffer 1 with water to be used for serial dilutions when preparing intermediate dilutions of the compounds and adding enzyme.

8. Prepare the Master Mix: **N wells** × (3.75 µl of 4X HMT assay buffer 1 (containing fresh DTT) + 1.25 µl of 20 µM S-adenosylmethionine + 7.5 µl of distilled water).

9. Add 12.5 µl of Master Mix to all the wells labeled "Positive Control", "Test Inhibitor" and "Blank".

10. For wells labeled "Substrate control", add 3.75 µl of 4X HMT assay buffer 1 + 8.75 µl of water.

	Blank	**Positive Control**	**Test Inhibitor**	**Substrate Control**
Master Mix	12.5 µl	12.5 µl	12.5 µl	-
4x HMT Assay Buffer 1 with DTT	-	-	-	3.75 µl
Water	-	-	-	8.75 µl
Test Inhibitor (compound)	-	-	2.5 µl	-
Diluent Solution (no inhibitor)	2.5 µl	2.5 µl	-	2.5 µl
1x HMT assay buffer 1	10 µl	-	-	-
SetDB1 (1 ng/µl)	-	10 µl	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

Prepare the test compound:

1. Prepare the Test inhibitor **(2.5 µl/well)**:

   a. For a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is **25 µl.**

   Without DMSO

   1.1. If the Test inhibitor is water-soluble, prepare serial dilutions in the in 1x HMT assay buffer (Step 1: #7), 10-fold more concentrated than the desired final concentrations.

   With DMSO

   1.2. If the Test inhibitor is soluble in DMSO

   a. Prepare a solution at 100-fold the highest desired concentration in DMSO, then dilute the compound 10-fold in in 1x HMT assay buffer (Step 1: #7) to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   b. Using in 1x HMT assay buffer (Step 1: #7) in 10% DMSO, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations to keep the concentration of DMSO constant.

   c. For the controls and the blank, prepare 10% DMSO in 1x HMT assay buffer (Step 1: #7) (vol/vol) so that all wells contain the same amount of DMSO (**Diluent Solution**).

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

11. Add 2.5 µl of Test inhibitor dilutions of each well designated "Test Inhibitor".

12. For "Positive Control", "Substrate Control" and "Blank", add 2.5 µl of the Diluent Solution (no inhibitor).

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

    a. Calculate the amount of SetDB1 enzyme required for your assay. Aliquot the remaining enzyme into single-use aliquots and store at -80°C immediately.

⚠ *Note: SetDB1 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme. Avoid multiple freeze/thaw cycles.*

    b. Dilute SetDB1 enzyme in 1x HMT Assay buffer 1 (Step 1: #7) to a concentration of 1 ng/µl (10 ng/10 µl). Keep the diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

⚠ *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.*

14. Add 10 µl of 1x HMT buffer 1 (Step 1: #7) to the well designated “Blank”.

15. Initiate the reaction by adding 10 µl of the diluted SetDB1 enzyme to the wells designated “Positive Control”, "Substrate Control", and "Test Inhibitor". Incubate at room temperature for 1 hour.

16. Remove the supernatant from the wells and wash three times with 100 µl of TBST buffer Blot dry onto clean paper towels.

17. Add 50 µl of Blocking buffer 4 to every well. Shake on a rotating platform for 10 min. Remove the buffer by inverting the plate and blot dry onto clean paper towels.

Step 2

1. Dilute the Primary antibody 100-fold with Blocking buffer 4.

    a. Add 100 µl per well.

    b. Incubate 1 hour at room temperature with slow shaking.

2. Wash three times with 100 µl of TBST buffer. Blot dry onto clean paper towels.

    a. Add 50 µl of Blocking buffer 4 to every well and incubate on a rotating platform for 10 min.

    b. Remove the Blocking buffer by inverting the plate and blot dry onto clean paper towels.

Step 3

1. Dilute the Secondary HRP-labeled antibody 1,000-fold with Blocking buffer 4.

    a. Add 50 µl per well.

    b. Incubate for 30 minutes at room temperature with slow shaking.

2. Wash strip three times with 100 µl TBST buffer.

    a. Add 50 µl of Blocking buffer 4 to every well and incubate on a rotating platform for 10 min.

    b. Remove the Blocking buffer by inverting the plate and blot dry onto clean paper towels.

3. On ice, mix 25 µl of HRP chemiluminescent substrate A and 25 µl of HRP chemiluminescent substrate B and **immediately** add 50 µl per well.

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

4. Immediately read the plate in a luminometer or 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 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

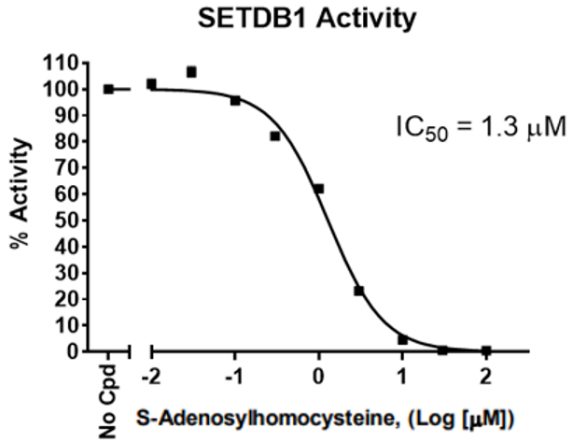

Figure 1: SetDB1 enzymatic activity.
Enzyme activity was measured using the SetDB1 Chemiluminescent Assay Kit (BPS Bioscience #51056) in the presence of increasing concentrations of inhibitor S-Adenosylhomocysteine (SAH). Luminescence was measured using a Bio-Tek fluorescent microplate reader.

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 SC, *et a*l. (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	51056-1	96 reactions
DNMT1 Chemiluminescent Assay Kit	52050L	96 reactions