Description

The Set8 Chemiluminescent Assay Kit is an ELISA (enzyme-linked immunosorbent assay)-based assay designed to measure the histone methylase activity of Set8 (SET domain containing lysine methyltransferase 8) for screening and profiling applications. The Set8 assay kit comes in a convenient 96-well format, with enough recombinant purified Set8 enzyme (amino acids 195-252), substrate, antibodies, and all the reagents necessary for detection.

Background

SET8 (PR/SET domain containing protein 8), also known as KMT5A (lysine-specific methylates 5A), belongs to the family of proteins that contain an SET domain. It has the specificity of being the only protein that monomethylates H4K20me1, but also participates in monomethylating proteins like p53 and PCNA (proliferating cell nuclear antigen). It is involved in cell cycle regulation and DNA replication and repair. Abnormal expression of SET8 can lead to cancer, and this protein is found at high levels in several cancers, such as breast cancer. Inhibition of SET8 was found to increase cancer cell sensitivity to radiotherapy. The use of inhibitors alone or in combinatory therapy promises to bring benefits in cancer therapy.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
51008	Set8, Active, GST-Tag*	90 µg	-80°C
52120	400 µM S-adenosylmethionine	4 x 250 µl	-80°C
52190	4x HMT Buffer 4	3 ml	-20°C
	Primary Antibody 23	12.5 µl	-80°C
52131H	Secondary HRP-Labeled Antibody 2	10 µl	-80°C
52100	Blocking Buffer 4	50 ml	+4°C
	0.5M DTT	2 x 200 µl	-20°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	+4°C
	ELISA ECL Substrate B (brown bottle)	6 ml	+4°C
	White plate (or strips) pre-coated with Histone-Substrate	1 plate	Room Temp

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

Materials Required but Not Supplied

- TBST Buffer (1x Tris-buffered saline, pH 8, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

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Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the 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

The Set8 Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration. Avoid the use of strong acids and bases, ionic detergents and high salt concentrations.

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 protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Ryuvidine as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1

1. Rehydrate each pre-coated well with 200 µl of TBST Buffer.

2. Incubate 15 minutes at Room Temperature (RT).

3. Tap the plate onto clean paper towel to remove the liquid.

4. Thaw S-adenosylmethionine on ice. Upon first thaw, briefly spin tube to recover its full content.

   Note: Aliquot S-adenosylmethionine into single use aliquots (minimum volume of 5 µl/aliquot). Store remaining S-adenosylmethionine in aliquots at -80°C immediately.

5. Add 240 µl of 0.5 M DTT to 4x HMT Buffer 4.

6. Dilute 4x HMT Buffer 4 with DTT 4-fold with distilled water. This makes 1x HTMT Buffer.

7. 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.

   7.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration.

   For the positive and negative controls, use TBST Buffer (Diluent Solution).

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OR

7.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with TBST Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in TBST Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in TBST 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%.

8. Prepare a Master Mixture (25 µl/well, except "Substrate Control" wells): N wells x (7.5 µl of 4x HMT Buffer 4 with DTT + 8.75 µl of 400 µM S-adenosylmethionine + 8.75 µl of distilled water).

9. Thaw **Set8** on ice. Briefly spin the tube containing the enzyme to recover its full content.

10. Dilute Set8 to 45 ng/µl (20 µl/well) with 1x HTMT Buffer.

11. Add 5 µl of the inhibitor solution to each well designated "Test Inhibitor".

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

13. Add 25 µl of Master Mixture to each well, except the "Substrate Control" wells.

14. Prepare a Deficient Master Mix (25 µl/"Substrate Control" well): N wells x (7.5 µl of 4x HMT Buffer 4 with DTT + 17.5 µl of distilled water).

15. Add 25 µl of Deficient Master Mix to the "Substrate Control" wells.

16. Initiate reaction by adding 20 µl of diluted Set8 to all wells, except "Blank" wells.

17. Add 20 µl of 1x HTMT Buffer to the "Blank" wells.

18. Incubate at RT for 1 hour.

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Component	Blank	Substrate Control	Positive Control	Test Inhibitor
Diluent Solution	5 µl	5 µl	5 µl	-
Test inhibitor	-	-	-	5 µl
Master Mix	-	25 µl	25 µl	25 µl
Deficient Master Mix	25 µl	-	-	-
Diluted Set8 (45 ng/µl)	-	20 µl	20 µl	20 µl
1x HTMT Buffer	20 µl	-	-	-
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

19. Wash the plate three times using 200 µl TBST Buffer per well.
20. Tap the plate onto clean paper towel to remove the liquid.
21. Add 100 µl of Blocking Buffer 4 into each well.
22. Incubate at RT for 10 minutes.
23. Tap the plate onto clean paper towel to remove the Blocking Solution.

Step 2:

1. Dilute 800-fold the Primary Antibody 23 with Blocking Buffer 4 (100 µl/well) .
2. Add 100 µl per well.
3. Incubate 1 hour at room temperature with slow agitation.
4. Wash plate three times with 200 µl TBST.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 µl of Blocking Buffer 4 into each well.
7. Incubate at RT for 10 minutes.
8. Tap the plate onto clean paper towel to remove the Blocking Solution.

Step 3:

1. Dilute 1000-fold the Secondary HRP-Labeled Antibody 2 with Blocking Buffer 4 (100 µl/well).
2. Add 100 µl of diluted Secondary Antibody 2 to each well.
3. Incubate 30 minutes at RT with slow agitation.
4. Tap the plate onto clean paper towel to remove the liquid.

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5. Add 100 µl of Blocking Buffer 4 into each well.

6. Incubate at RT for 10 minutes.

7. Tap the plate onto clean paper towel to remove the Blocking Solution.

Step 4:

1. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

2. Add 100 µl of mix per well.

3. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

4. The "Blank" value should be subtracted from all other values.

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. 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).

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Example Results

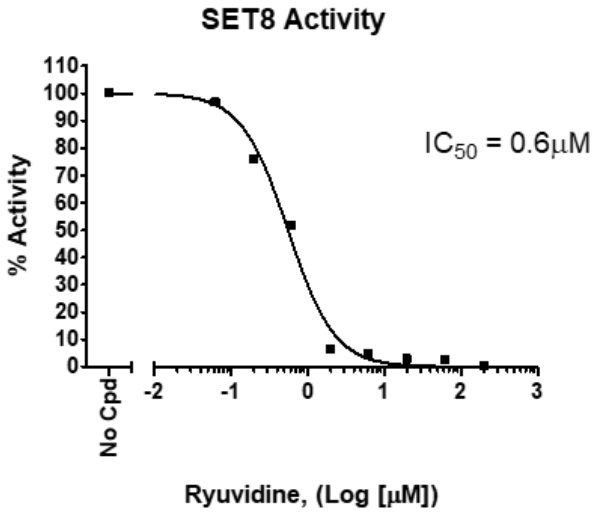

Figure 1: Inhibition of Set8 activity by Ryuvidine.
Set8 inhibition was measured in the presence of increasing concentrations of Ryuvidine. Luminescence was measured using a Bio-Tek microplate reader.

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

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Pan D., *et al.*, 2022 *Biomed Environ Sci* 35(3), 194:205.

Related Products

Products	*Catalog #*	*Size*
Anti-SET8 Polyclonal Antibody	25315	50 µg
DOT1L (KMT4) Chemiluminescent Assay Kit	52202	96 reactions
DOT1L (KMT4), GST-Tag Recombinant	51005	50 µg
SMYD3 Homogeneous Assay Kit	52063	384 reactions
SMYD3 (35-end), GST-Tag Recombinant	51015	20 µg
SMYD3 (full length), FLAG-Tag Recombinant	51016	20 µg

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