Description

The EZH2 Chemiluminescence Assay Kit is an EZH2/EED/SUZ12/RbAp48/AEBP2 chemiluminescence three step kit designed to measure activity of the EZH2 (enhancer of zeste homolog 2) complex (EZH2/EED/SUZ12/RbAp48/AEBP) for screening and profiling purposes. The EZH2 Assay Kit comes in a convenient format, with a 96-well plate precoated with histone H3 peptide substrate, and enough antibody against methylated K27 residue of Histone H3, secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified EZH2 complex for 100 enzyme reactions.

EZH2 Activity Assay Kit uses a highly specific antibody that recognizes methylated Histone H3 on K27. In a first step, S-adenosylmethionine is incubated with methyltransferase and sample prepared in assay buffer for one hour. This is followed by addition of the primary antibody. Finally, the plate is treated with an HRP-labeled secondary antibody and addition of the HRP substrate to produce chemiluminescence signal proportional to the EZH2 activity.

Background

EZH2 (enhancer of zeste homolog 2) is a histone-lysine N-methyltransferase enzyme, that acts by adding methyl groups to the lysine 27 (K27) of histone H3, making it a silent chromatin. It is a functional unit of the larger complex PRC2 (polycomb repressive complex 2), which also includes EED, Suz12, AEBP2 (adipocyte enhancer-binding protein) and RbAp48 (histone-binding protein RBBP4). PRC2 is crucial for epigenetic regulation and is involved in stem cell differentiation and embryonic development. Abnormalities in PRC2 result in cancer since this complex also promotes double strand DNA repair. EZH2 is seen as an attractive target in cancer therapy, as its levels are elevated in multiple cancer types (example, breast, renal cancer, melanoma, and lymphoma). The development of inhibitors for EZH2 and PRC2 is a promising area of research for the treatment of cancer.

Applications

Enzymatic kinetic studies and screen molecules that inhibit EHZ2 activity in drug discovery high throughput applications (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
51004	EZH2/EED/SUZ12/RbAp48/AEBP2, FLAG-Tag, His-Tag*	25 µg	-80°C
52120	400 µM S-adenosylmethionine	250 µl	-80°C
52140F	Primary Antibody 6	12.5 µl	-80°C
52131H	Secondary HRP-Labeled Antibody 2	10 µl	-80°C
52170	4x HMT Assay Buffer 2	3 ml	-20°C
79556	Blocking Buffer 1	50 ml	+4°C
	HRP Chemiluminescent Substrate A	6 ml	+4°C
	HRP Chemiluminescent Substrate B	6 ml	+4°C
	96-well plate precoated with histone substrate	1 plate	+4°C

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

Materials Required but Not Supplied

- TBST Buffer (1x TBS, pH 8 with 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Rotating or rocker platform

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 EHZ2 Assay Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

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

Step 1:

1. Rehydrate the microwells by adding 150 µl of TBST buffer to every well.

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

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

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

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

5. Prepare a Master Mix (25 µl/well, except "Substrate Control"): N wells × (7.5 µl of **4x HMT Assay Buffer** 2 + 1.25 µl of 400 µM S-adenosylmethionine + 16.25 µl of distilled water).

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

7. Prepare a Deficient Master Mix (25 µl/well for each "Substrate Control" well): N wells × (7.5 µl of **4x HMT Assay Buffer** 2 + 17.5 µl of distilled water).

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

9. Dilute 4-fold the 4x HMT Assay Buffer 2 with distilled water to generate 1x HMT Assay Buffer 2.

10. Prepare the Test Inhibitor/Activator (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 serial dilutions in the 1x HMT Assay Buffer 2, 10-fold more concentrated than the desired final concentrations.

    For the positive and negative controls, use 1x HMT Assay Buffer 2 (Diluent Solution).

    OR

    10.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x HMT Assay Buffer 2 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

    Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x HMT Assay Buffer 2 to keep the concentration of DMSO constant.

    For positive and negative controls, prepare 10% DMSO in water (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 inhibitor/activator solution to each well designated "Test Inhibitor/Activator".

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

13. Add 20 µl of 1x HMT Assay Buffer 2 to the "Blank" wells.

14. Thaw **EZH2 complex** on ice. Briefly spin the tube to recover its full content.

15. Dilute EZH2 with 1x HMT Assay Buffer 2 to 5-12.5 ng/µl (20 µl/well).

16. Initiate the reaction by adding 20 µl of diluted EZH2 to the wells labeled "Positive Control", "Test Inhibitor", and "Substrate Control".

17. Incubate the reaction at RT for one hour.

	Test Inhibitor	Substrate Control	Positive Control	Blank
Master Mix	25 µl	-	25 µl	25 µl
Deficient Master Mix	-	25 µl	-	-
Test Inhibitor/Activator	5 µl	–	–	-
Diluent Solution	–	5 µl	5 µl	5 µl
1x HMT Assay Buffer 2	–	-	–	20 µl
Diluted EZH2 (5-12.5 ng/µl)	20 µl	20 µl	20 µl	-
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

18. Wash the plate three times with 200 µl TBST Buffer per well.
19. Blot dry onto clean paper towels between washes.
20. Add 100 µl of **Blocking Buffer 1** to every well.
21. Incubate for 10 minutes with gentle agitation.
22. Remove supernatant by drying onto clean paper.

Step 2:

1. Dilute 800-fold the **Primary Antibody 6** with Blocking Buffer 1.
2. Add 100 µl of diluted Primary Antibody 6 per well.
3. Incubate 1 hour at RT with gentle agitation.
4. Wash the plate three times with 200 µl TBST Buffer per well.
5. Blot dry onto clean paper towels between washes.
6. Add 100 µl of Blocking Buffer 1 to every well.
7. Incubate for 10 minutes with gentle agitation.
8. Remove supernatant by drying onto clean paper.

Step 3:

1. Dilute 1,000-fold the **Secondary HRP-labeled Antibody 2** with Blocking Buffer 1.
2. Add 100 µl per well.
3. Agitate on a rotator platform for 30 minutes at RT.

4. Wash the plate three times with 200 µl TBST Buffer per well.
5. Blot dry onto clean paper towels between washes.
6. Add 100 µl of Blocking Buffer 1 to every well.
7. Incubate for 10 minutes with gentle agitation.
8. Remove supernatant by drying onto clean paper.
9. Just before use, mix on ice 50 µl HRP Chemiluminescent Substrate A and 50 µl HRP Chemiluminescent Substrate B (100 µl of mix/well).
10. Add 100 µl of mix per well.
11. Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.
12. 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).

Example Results

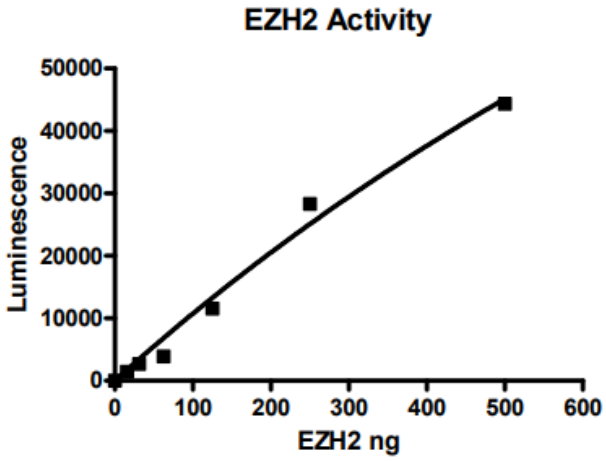

Figure 1: EZH2/EED/SUZ12/RbAp48/AEBP2 enzyme activity.
EZH2 complex activity was with increasing amounts of EZH2 complex. Luminescence was measured using a Bio-Tek fluorescent microplate reader.

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

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

Related Products

Products	*Catalog #*	*Size*
EZH2 Homogeneous Assay Kit	52059	384 reactions
EZH2/EED inactive, His-Tag, FLAG-Tag Recombinant	51002	20 µg
EZH2/EED/SUZ12, His-Tag, FLAG-Tag Recombinant	51003	50 µg
EZH2/EED/SUZ12/RbAp48/AEBP2, FLAG-Tag, His-Tag Recombinant	51004	50 µg

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