EZH2 Homogeneous Assay Kit

#52059
384 reactions

Description

The EZH2 Homogenous Assay Kit is 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 Homogeneous Assay Kit comes in a convenient format 384-well AlphaLISA® format, with enough purified recombinant EZH2 complex, S-adenosylmethionine, primary antibody, substrate and buffers for 400 enzyme reactions.

The EZH2 Homogeneous Activity Assay Kit uses a highly specific antibody. In a first step, the methyltransferase is incubated with the substrate for one hour. This is followed by addition of the primary antibody and acceptor beads. Finally, the donor beads are added, and Alpha-counts are measured. The Alpha-counts are 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*	2 x 25 µg	-80°C
52120	20 µM S-adenosylmethionine	2 x 250 µl	-80°C
52140F	Primary Antibody 6	20 µl	-20°C
	Histone Octamer Substrate	400 µl	-80°C
52170-A	4x HMT Assay Buffer 2A	3 ml	-20°C
	4x Detection Buffer 2	2 ml	-20°C

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

Materials Required but Not Supplied

Name	Catalog #
AlphaLISA® Anti-Rabbit IgG Acceptor Beads, 5 mg/ml	Perkin Elmer #AL104C
AlphaScreen® Nickel Donor Beads, 5 mg/ml	Perkin Elmer #AS101D
Optiplate - 384	Perkin Elmer #6007290
AlphaScreen® microplate reader	

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 EZH2 Homogenous Assay Kit is compatible with up to 1% final DMSO concentration.
- Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (λ=520-620 nm), such as Trypan Blue.
- Avoid the use of potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}).
- The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. Media like MEM, which lacks these components, does not affect AlphaScreen assays.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor".
- Depending on the nature of the inhibitor, pre-incubation of the enzyme with the inhibitor may be necessary.
- We recommend using GSK343 as an internal control for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1:

1. Dilute 4-fold the 4x HMT Assay Buffer 2A with distilled water. This makes 1x HMT Assay Buffer 2A.

2. Prepare the Test Inhibitor/Activator (3 µl/well): for a titration prepare serial dilutions at concentrations 3.3-fold higher than the desired final concentrations. The final volume of the reaction is 10 µl.

   2.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x HMT Assay Buffer 2A, 3.3-fold more concentrated than the desired final concentrations.

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

   OR

2.2 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 30-fold in 1x HMT Assay Buffer 2A to prepare the highest concentration of the 3.3-fold intermediate dilutions. The concentration of DMSO is now 3.3%.

Prepare serial dilutions of the Test Inhibitor at 3.3-fold the desired final concentrations using 3.3% DMSO in 1x HMT Assay Buffer 2A to keep the concentration of DMSO constant.

For positive and negative controls, prepare 3.3% 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%.

3. Add 3 µl of inhibitor/activator solution to each well designated "Test Inhibitor/Activator".
4. Add 3 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
5. Thaw **EZH2 complex** on ice. Briefly spin the tube to recover its full content.
6. Dilute EZH2 with 1x HMT Assay Buffer 2A to 60 ng/µl (2 µl/well).
7. Add 2 µl of diluted EZH2 to the wells labeled "Positive Control" and "Test Inhibitor/Activator".
8. Add 2 µl of 1x HMT Assay Buffer 2A to the "Blank" wells.
9. Pre-incubate the reaction at Room Temperature (RT) for 30 minutes with slow agitation.
10. 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.

11. Prepare a Master Mix (5 µl/well): N wells × (2 µl of **4x HMT Assay Buffer** 2A + 1 µl of 20 µM S-adenosylmethionine + 1 µl of Histone Octamer Substrate + 1 µl of distilled water).
12. Add 5 µl of Master Mix to each well.
13. Incubate at RT for 1 hour with slow agitation.

Protect your samples from direct exposure to light for step 2 and 3. Photobleaching will occur!

	Test Inhibitor/Activator	Positive Control	Blank
Inhibitor/Activator Mix	3 µl	-	-
Diluent Solution	-	3 µl	3 µl
Diluted EZH2 (60 ng/µl)	2 µl	2 µl	-
1x HMT Assay Buffer 2A	–	-	2 µl
30 minutes at RT			
Master Mix	5 µl	5 µl	5 µl
Total	**10 µl**	**10 µl**	**10 µl**

Step 2:

1. Dilute 4-fold the 4x Detection Buffer 2 with distilled water.
2. Dilute 250-fold the **Primary Antibody 6** and 500-fold the AlphaLISA® Anti-Rabbit IgG Acceptor Beads together in 1x Detection Buffer 2 (10 µl of mix per well).
3. Add 10 µl of diluted mix to each well.
4. Incubate 30 minutes at RT.

Step 3:

1. Dilute 125-fold the AlphaScreen® Nickel Donor Beads with 1x Detection Buffer 2 (10 µl/well).
2. Add 10 µl to each well.
3. Incubate for 60 minutes at RT.
4. Read Alpha-Counts.
5. The "Blank" control might be important to determine the background A-screen counts in the assay. The blank value should be subtracted from all other values.

Example Results

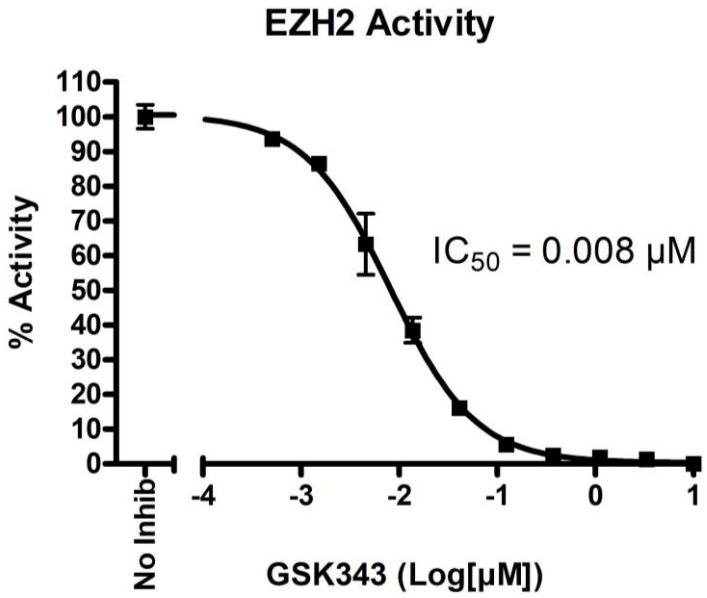

Figure 1: EZH2/EED/SUZ12/RbAp48/AEBP2 activity is inhibited by GSK343.
EZH2 complex activity was measured with increasing amounts GSK343.

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 Chemiluminescence Assay Kit	52009L	96 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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