6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
EZH2 Assay Kit
Catalog # 52009L

DESCRIPTION: The *EZH2/EED/SUZ12/RbAp48/AEBP2 Direct Activity Assay Kit (EZH2 Assay Kit)* is designed to measure activity of the EZH2 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, an antibody against methylated K27 residue of Histone H3, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified EZH2 complex for 100 enzyme reactions. The key to the EZH2 Direct Activity Assay Kit is a highly specific antibody that recognizes methylated Histone H3K27. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme for one hour. Next, primary antibody is added. Finally, the plate is treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51004	EZH2/EED/SUZ12/RbAp48/AEBP2	25 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
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	
79670	ELISA ECL substrate (2 components)	6 ml each	Room Temp	
	96-well plate precoated with histone substrate	1 plate	+4°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Rotating or rocker platform

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APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCES:
Dillon SC, Zhang X, Trievel RC, Cheng X. Genome Biology 2005; **6:**227.
Morin, RD., et al. Nat Genet. 2010, **42**(2):181.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 150 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the plate onto clean paper towels to remove liquid.

2) Thaw **S-adenosylmethionine** on ice. Upon first thaw, briefly spin tube containing **S-adenosylmethionine** to recover full content of the tube. Aliquot **S-adenosylmethionine** into single use aliquots. Store remaining **S-adenosylmethionine** in aliquots at -80ºC immediately. *Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

3) Prepare the master mixture: N wells × (7.5 µl **4x HMT assay buffer 2** + 1.25 µl **400 µM S-adenosylmethionine** + 16.25 µl **H_2O**)

4) Add 25 µl of master mixture to each well designated for the “Positive Control”, “Test Inhibitor”, and “Blank”. For the “Substrate Control”, add 7.5 µl **4x HMT assay buffer 2** + 17.5 µl **H_2O**

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	Blank	Substrate Control	Positive Control	Test Inhibitor
4x HMT assay buffer 2	7.5 µl	7.5 µl	7.5 µl	7.5 µl
400 µM S-adenosylmethionine	1.25 µl	–	1.25 µl	1.25 µl
H_2O	16.25 µl	17.5 µl	16.25 µl	16.25 µl
Test Inhibitor/Activator	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1x HMT assay buffer 2	20 µl	–	–	–
EZH2 (5-12.5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

5) Add 5 µl of inhibitor solution of each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer).

6) Add 20 µl of **1x HMT assay buffer 2** to the well designated "Blank".

7) Thaw **EZH2 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **EZH2 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **EZH2 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme. Perform this step after all other dilutions and immediately prior to initiating reactions.*

8) Dilute **EZH2 enzyme** in **1x HMT assay buffer 2** at 5-12.5 ng/µl (100-250 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

9) Initiate reaction by adding 20 µl of diluted **EZH2** prepared as described above. Incubate at room temperature for one hour.

10) Wash the plate three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

11) Add 100 µl of **Blocking buffer 1** to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

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Step 2:

1) Dilute "**Primary antibody 6**" 800-fold with **Blocking buffer 1**.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash plate three times with TBST buffer and incubate in **Blocking buffer 1** as in steps 1-10 and 1-11.

Step 3:

1) Dilute "**Secondary HRP-labeled antibody 2**" 1,000-fold with **Blocking buffer 1**.

2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash plate with TBST buffer and incubate in **Blocking buffer 1** as in step 1-10 and 1-11.

4) Just before use, mix on ice 50 µl **ELISA ECL substrate A** and 50 µl **ELISA ECL substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

5) Immediately read sample in a luminometer or microtiter-plate 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 wave length 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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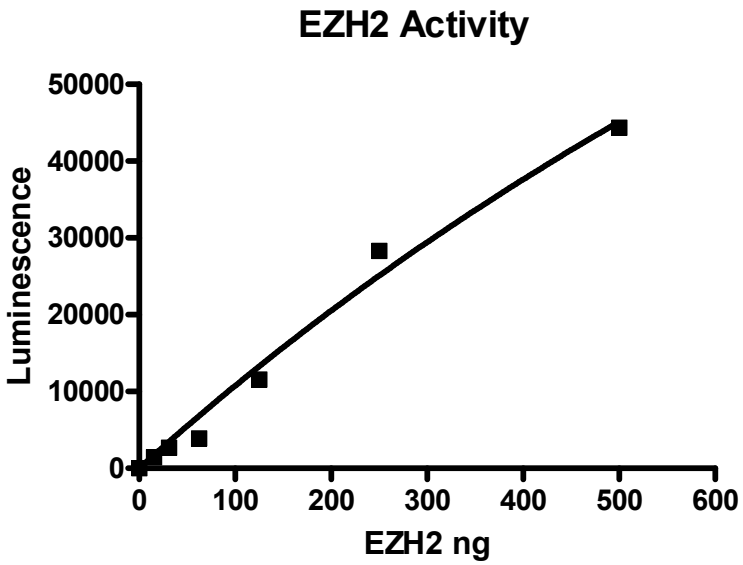
Example of Assay Results:



EZH2/EED/SUZ12/RbAp48/AEBP2 enzyme activity, measured using the EZH2 Direct Assay Kit, BPS Bioscience # 52009L. Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS	CATALOG #	SIZE
EZH1/EED/SUZ12	51006	50 µg
EZH1/EED/SUZ12/RbAp48/AEBP2	51007	50 µg
EZH2/EED inactive	51002	20 µg
EZH2/EED/SUZ12	51003	50 µg
EZH2/EED/SUZ12/RbAp48/AEBP2	51004	50 µg
SUV39H1 Assay Kit	52006	96 reactions
SUV39H2 Assay Kit	52007	96 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	EZH2 Complex has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh EZH2 Complex, BPS Bioscience #51004. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of EZH2 Complex, BPS Bioscience #51004 to create a standard curve.