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Data Sheet

EZH2 Homogeneous Assay Kit

Catalog #52059

DESCRIPTION: The *EZH2 Homogeneous Assay Kit* is designed to measure EZH2 activity for screening and profiling applications. EZH2 is a histone methyltransferase that exhibits methylation activity toward H3-K27. The *EZH2 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with histone substrate mixture, primary antibody, methylation assay buffer, and purified EZH2 for 384 enzyme reactions. The key to the *EZH2 Homogeneous Assay Kit* is a highly specific antibody that recognizes methylated substrate. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, a sample containing EZH2 enzyme is incubated with the histone substrate for one hour. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog. #	Component	Amount	Storage	Storage
51004	EZH2	25 µg	-80°C	***(Avoid freeze/thaw cycles!)***
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	

MATERIALS REQUIRED BUT NOT SUPPLIED:
AlphaLISA anti-rIgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
AlphaScreen Nickel donor beads, 5 mg/ml (PerkinElmer #AS101D)
Optiplate -384 (PerkinElmer #6007290)
AlphaScreen microplate reader

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions ($Fe2^+$, $Fe3^+$, $Cu2^+$, $Zn2^+$ and $Ni2^+$). 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. MEM, which lacks these components, does not affect AlphaScreen assays.

STABILITY: At least one year from date of receipt, when stored as directed.

ASSAY PROTOCOL:

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All samples and controls should be tested in duplicate. We recommend preincubating the enzyme with inhibitor, however, it is acceptable to add the substrate mixture and inhibitor followed by diluted EZH2 without the preincubation step.

Step 1:

1) Dilute **4x HMT assay buffer 2A** four-fold to prepare **1x HMT assay buffer 2A.** Prepare only the amount necessary for your current experiment.

2) Prepare serial dilutions of the test inhibitors in **1x HMT assay buffer 2A**. Add 3 µl of inhibitor solution to each well designated "Test Sample". For the wells designated "Blank" and "Positive Control" add 3 µl of the same solution without inhibitor or "Inhibitor buffer" (typically **1x HMT assay buffer 2A** with respective concentration of DMSO).

3) Thaw **EZH2** 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. *Note: EZH2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **EZH2** in **1X HMT assay buffer** at 30 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

5) Preincubate 2 µl of diluted **EZH2** with 3 µl of diluted inhibitor(s) for up to 30 minutes at room temperature, with slow shaking. For the wells designated as "Blank", add 2 µl **1x HMT assay buffer 2A**.

	Blank	Positive Control	Test Sample
EZH2 (30 ng/µl)	–	2 µl	2 µl
1x HMT assay buffer 2A	2 µl	–	–
Test Inhibitor/Activator	–	–	3 µl
Inhibitor buffer*	3 µl	3 µl	–
4x HMT assay buffer 2A	2 µl	2 µl	2 µl
Histone octamer substrate	1 µl	1 µl	1 µl
20 µM S-adenosylmethionine	1 µl	1 µl	1 µl
Distilled water	1 µl	1 µl	1 µl
Total	**10 µl**	**10 µl**	**10 µl**

*) Inhibitor buffer typically represents 1x HMT assay buffer 2A with proper concentration of DMSO.

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6) Prepare master mix: N wells × (2 µl **4x HMT assay buffer 2A** + 1 µl **histone octamer substrate** + 1 µl **20 µM S-adenosylmethionine** + 1 µl **distilled water**).

7) Initiate reaction by adding 5 µl of of master mixture to each well. Incubate at room temperature for one hour. *Note: All incubations are performed with slow shaking on a rotator platform.*

Step 2:

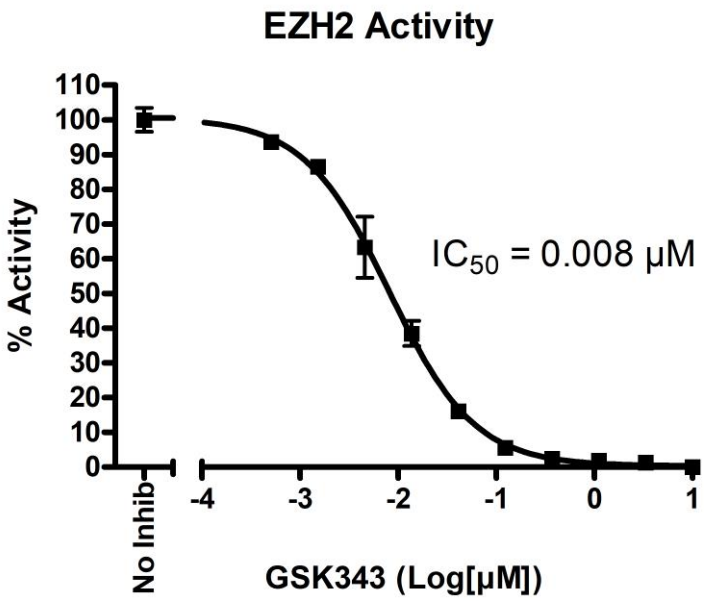
Note: Protect your samples from direct exposure to light!

1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) (1:500) and Primary antibody 6 (1:250) with 1x Detection buffer in one step. Add 10 µl of acceptor beads/antibody mixture per well. Incubate 30 min at room temperature.

Step 3:

1) Dilute AlphaScreen Nickel donor beads (PerkinElmer #AS101D) 125-fold with 1x Detection buffer. Add 10 µl per well. Incubate for 60 min. at room temperature.
2) Read Alpha-counts.

Example of Assay Results:



EZH2 enzyme activity, measured using the EZH2 Homogeneous Assay Kit, BPS Bioscience Cat. #52059. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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REFERENCES:

Dillon, S.C., *et al.* 2005. *Genome Biology* **6:**227.

RELATED PRODUCTS:

EZH1/EED/SUZ12 Recombinant Protein	#51006	50 µg
EZH1/EED/SUZ12/RbAp48/AEBP2 Complex, Recomb.	#51007	50 µg
EZH2/EED inactive Recombinant Protein	#51002	20 µg
EZH2/EED/SUZ12 Recombinant Protein	#51003	50 µg
EZH2/EED/SUZ12/RbAp48/AEBP2 Complex, Recomb.	#51004	50 µg
EZH2 Chemiluminescent Assay Kit	#52009L	96 reactions
EZH2 (Y641F) Chemiluminescent Assay Kit	#52075	96 reactions
EZH2 (Y641N) Chemiluminescent Assay Kit	#52076	96 reactions
EZH1 Chemiluminescent Assay Kit	#52079	96 reactions