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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 (his-tagged), 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	100 µg	-80°C	(Avoid freeze/thaw cycles!)
52120-A	100 µM S-adenosylmethionine	400 µl	-80°C	
52140H	Primary antibody 8*	5 µl	4°C	
	Histone mixture (his-tagged)	400 µl	-80°C	
52170-A	4x HMT assay buffer 2	3 ml	-20°C	
	4x Detection buffer 2	2 ml	-20°C	

MATERIALS REQUIRED BUT NOT SUPPLIED:

AlphaLISA anti-rlgG 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 (NaN₃) 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.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) 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.

2) Dilute EZH2 in 1X HMT assay buffer at 100 ng/μl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

3) Using master mixes as much as possible, add the following reagents to the microwells, in duplicate:

	Blank	Substrate Control	Positive Control	Test Sample
EZH2 (100 ng/μl)	–	2.5 μl	2.5 μl	2.5 μl
4x HMT assay buffer 2	2.5 μl	2.5 μl	2.5 μl	2.5 μl
100 μM S-adenosylmethionine	1 μl	–	1 μl	1 μl
Histone substrate	1 μl	1 μl	1 μl	1 μl
Test Inhibitor/Activator	–	–	–	X μl
H ₂ O	5.5 μl	4 μl	3 μl	3 – X μl
Total	10 μl	10 μl	10 μl	10 μl

4) Add the entire reaction mixture (10 μl) to the well of a 384-well white plate. Incubate at room temperature for 1 hour.

Step 2:

Note: Protect your samples from direct exposure to light!

- 1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #6760607C or #AL104C) 150-fold with 1x Detection buffer. Add 5 μl per well. Shake plate briefly.
- 2) Dilute "Primary antibody 8" 400-fold with 1x Detection buffer. Add 5 μl per well. Shake plate. Incubate 30 min at room temperature.

Step 3:

- 1) Dilute AlphaScreen Nickel donor beads (PE #AS101D) 100-fold with 1x Detection buffer. Add 10 μl per well. Incubate for 30-60 min. at room temperature.
- 2) Read Alpha-counts.

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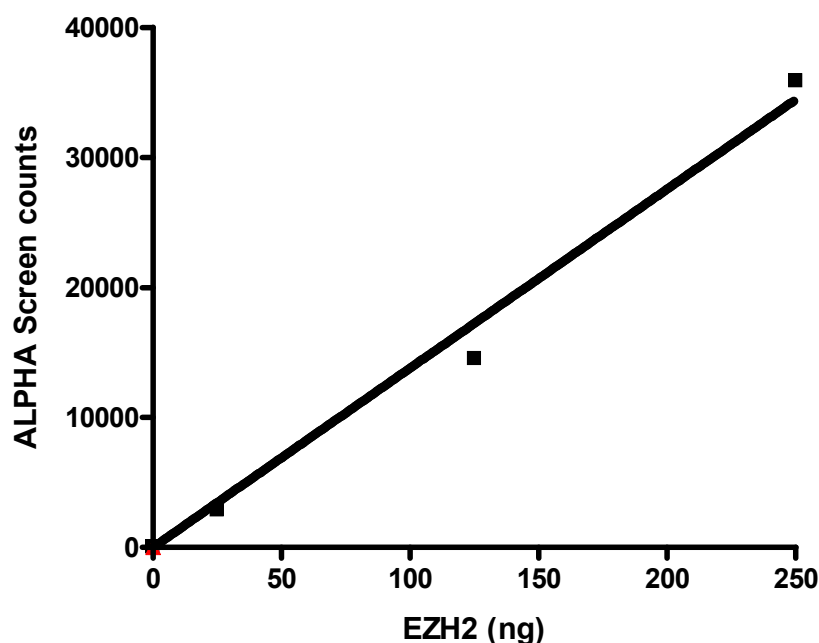
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Example of Assay Results:

EZH2 activity (ALPHA Screen)



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

REFERENCES:

Dillon SC, 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

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