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Data Sheet

EZH2-EED Binding Assay Kit

Catalog # 52066
Size: 384 reactions

DESCRIPTION: The EZH2-EED Binding Assay Kit is designed to measure the inhibition of the protein-protein interaction between EZH2 and EED. The kit comes in a convenient AlphaLISA® format, with GST-tagged EZH2, FLAG-tagged EED, and enough assay buffer to perform a total of 384 reactions. The key to this kit is the specific binding of EZH2 to EED. With this kit, only two simple steps on a microtiter plate are required. First, incubate a sample containing EZH2, EED and an inhibitor of choice. Next, incubate with a mixture of acceptor and donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50279	EZH2, GST-tag	20 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
50280	EED, FLAG-tag	10 µg	-80°C	
52170-A	4x HMT Assay Buffer 2A	4 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Anti-FLAG AlphaLISA® Acceptor Beads Beads, 5 mg/ml (PerkinElmer #AL112C)
AlphaScreen® Glutathione Donor Beads, 5 mg/ml (PerkinElmer #6765300)
Optiplate-384 (PerkinElmer #6007290)
AlphaScreen® microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for the study of EZH2 binding assays, screening inhibitors and selectivity profiling.

CONTRAINDICATIONS: DMSO concentrations above 0.5%. 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 (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. MEM, which lacks these components, does not affect AlphaScreen® assays.

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

REFERENCE: Kong, X., *et al., J. Med. Chem.* 2014; **57**:9512.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

Step 1:

1) Prepare **1x HMT Assay Buffer 2A** by diluting one part **4x HMT Assay Buffer 2A** with three parts distilled water. Prepare only enough **1x HMT Assay Buffer 2A** required for the assay.

2) Thaw **EZH2 and EED** on ice. Upon first thaw, briefly spin tube containing protein to recover full content of the tube. Aliquot protein into single use aliquots. Store remaining undiluted protein in aliquots at -80°C immediately. *Note:* both proteins are *very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

3) Dilute **EZH2** in **1x HMT Assay Buffer 2A** at 9 ng/µl. Add 4 µl per well. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

4) Add 2 µl of inhibitor solution to each well designated "Test Inhibitor". Add 2 µl of inhibitor buffer to each well designated for "Blank" and "Positive Control" (the same solution without inhibitor). *Note: Keep DMSO concentration below 0.5 %.*

5) Add 4 µl of **1x HMT Assay Buffer 2A** to the wells designated as "Blank".

6) Dilute **EED** in **1x HMT Assay Buffer 2A** at 2 ng/µl. Initiate reaction by adding 4 µl per well designated "Positive Control" and "Test Inhibitor". Keep diluted protein on ice until use. Discard any unused diluted protein after use. Incubate at room temperature for 60 minutes.

	Blank	Positive Control	Test Inhibitor
EZH2 (9 ng/µl)	4 µl	4 µl	4 µl
Test Inhibitor	–	–	2 µl
Inhibitor buffer (no inhibitor)	2 µl	2 µl	–
1x assay buffer	4 µl	–	–
EED (2 ng/µl)	–	4 µl	4 µl
Total	**10 µl**	**10 µl**	**10 µl**

Step 2:
Note: Protect your samples from direct exposure to light!

1) Dilute Anti-FLAG Acceptor Beads 500-fold with 1x assay buffer. Dilute Glutathione donor beads 125-fold in the same solution. Add 20 µl per well. Shake plate briefly. Incubate at room temperature for 60 minutes.

2) Read Alpha-counts.

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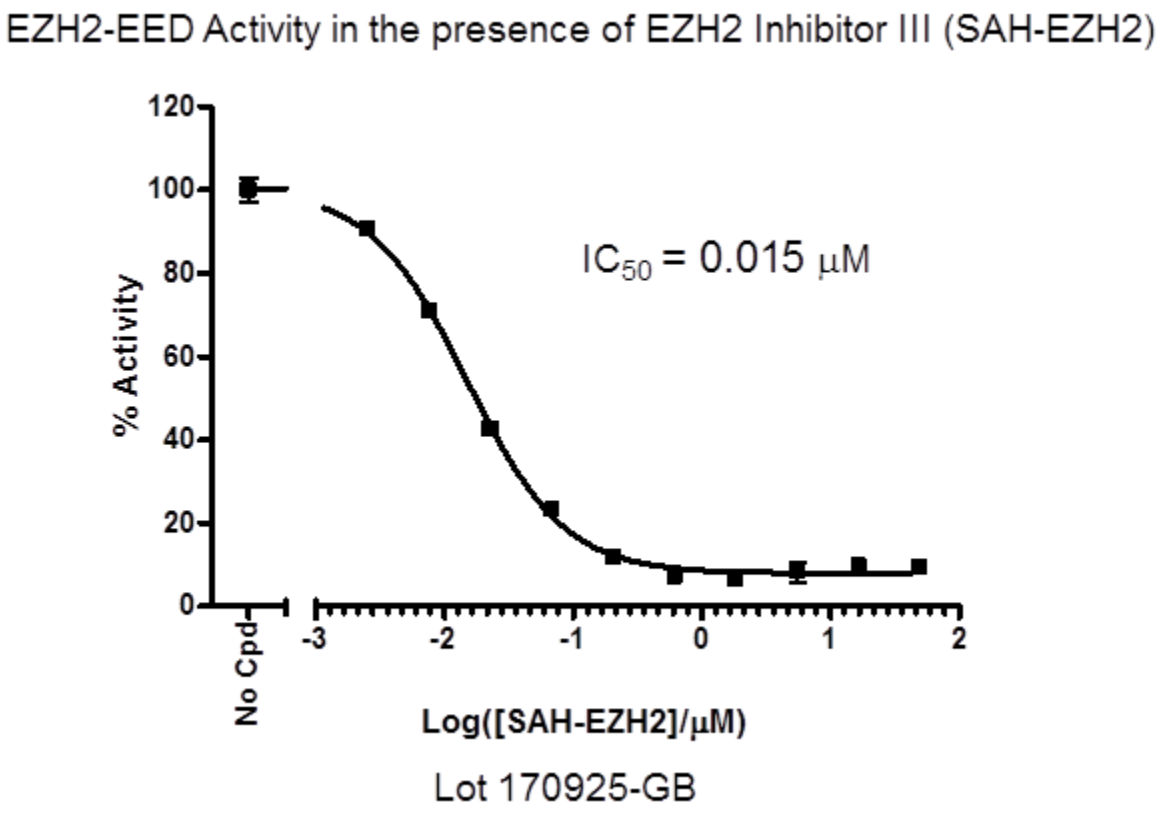
Due to lot to lot variability in AlphaScreen® *bead performance, it may be necessary to optimize assay conditions. For example, slight adjustments to EZH2 or EED concentrations may improve signal-to-noise ratio.*

EXAMPLE OF ASSAY RESULTS:



Assay was done using AlphaLISA format to measure the inhibition of the protein-protein interaction between EZH2 and EED. Reaction was performed at room temperature for 1 hour. Anti-FLAG Acceptor Beads and Glutathione Donor Beads were added and the reaction was incubated for 1 hour at room temperature. Alpha Counts were read on AlphaScreen microplate reader.

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RELATED PRODUCTS:

Product Name	Catalog #	Size
EED	50280	50 µg
EZH2 (non-complexed)	50279	20 µg
EZH2/EED (inactive)	51002	20 µg
EZH2/EED/SUZ12	51003	50 µg
EZH2/EED/SUZ12/RbAp48/AEBP2	51004	50 µg
EZH1/EED/SUZ12/RbAp48/AEBP2	51007	50 µg
EZH2 (Y641F)/EED/SUZ12/RbAp48/AEBP2	51017	20 µg
EZH2 Homogeneous Assay Kit	52059	384 reactions
EZH2 Chemiluminescent Assay Kit	52009L	96 reactions
EZH2 (Y641F) Chemiluminescent Assay Kit	52075	96 reactions
EZH2 (A677G) Chemiluminescent Assay Kit	52077	96 reactions