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Data Sheet

EZH2 (Y641N) Chemiluminescent Assay Kit

Catalog # 79277
Size: 384 reactions

DESCRIPTION: The *EZH2 (Y641N) Chemiluminescent Assay Kit* is designed to measure activity of the mutant EZH2 complex (EZH2 (Y641N)/EED/SUZ12/RbAp48/ AEBP) for screening and profiling purposes. The *EZH2 (Y641N) Chemiluminescent Assay Kit* comes in a convenient format, with wells 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 (Y641N) complex for 384 enzyme reactions. The key to the EZH2 (Y641N) 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 strips are 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	
51028	EZH2 (Y641N)/EED/SUZ12/RbAp48/AEBP2	20 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120	20 µM S-adenosylmethionine	4x250 µl	-80°C	
52140X	Primary Antibody 24	25 µl	-80°C	
52131H	Secondary HRP-labeled Antibody 2	20 µl	-80°C	
52170B	4x HMT Assay Buffer 2B	2x3 ml	-20°C	
52100	Blocking Buffer	2x50 ml	+4°C	
	HRP chemiluminescent substrate A (translucent bottle)	2x6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	2x6 ml	+4°C	
	384-well plate precoated with histone substrate	1 plate	+4°C	

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1x Tris-buffered saline, pH 8.0, containing 0.05% Tween-20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Rotating or rocker platform

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

REFERENCE(S):

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

2. Morin, R.D., *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 90 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-adenosyl-methionine** 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 **4× HMT Assay Buffer 2B** + 2.5 µl **20 µM S-adenosylmethionine** + 15 µl **H_2O**)

	Blank	Substrate Control	Positive Control	Test Inhibitor
4× HMT assay buffer 2B	7.5 µl	7.5 µl	7.5 µl	7.5 µl
20 µM S-adenosylmethionine	2.5 µl	–	2.5 µl	2.5 µl
H_2O	15 µl	17.5 µl	15 µl	15 µl
Test Inhibitor/Activator	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1× HMT assay buffer 2B	20 µl	–	–	–
EZH2(Y641N) (1-2 ng/µl)	–	20 µl	20 µl	20 µl

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Total	50 µl	50 µl	50 µl	50 µl

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 **4× HMT Assay Buffer 2** + 17.5 µl **H_2O**

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 **1 × HMT assay buffer 2B** to the well designated "Blank".

7) Thaw **EZH2(Y641N) enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **EZH2(Y641N) enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -70°C immediately. *Note: **EZH2(Y641N) enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

8) Dilute **EZH2(Y641N) enzyme** in **1× HMT assay buffer 2B** at 1-2 ng/µl (20-40 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(Y641N)** prepared as described above. Incubate at room temperature for one hour.

10) Wash the strips three times with 90 µl TBST buffer. Blot dry onto clean paper towels.

11) Add 50 µl of **Blocking Buffer** to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

Step 2:

1) Dilute **Primary antibody 24** 800-fold with **Blocking Buffer**.

2) Add 50 µl per well. Incubate 1 hour at room temperature with slow shaking.

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

Step 3:

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

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2) Add 50 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash the strips with TBST buffer and incubate in **Blocking Buffer** as described in step 1-10 and 1-11.

4) Just before use, mix on ice 25 µl **HRP chemiluminescent substrate A** and 25 µl **HRP chemiluminescent substrate B** and add 50 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 wavenlength 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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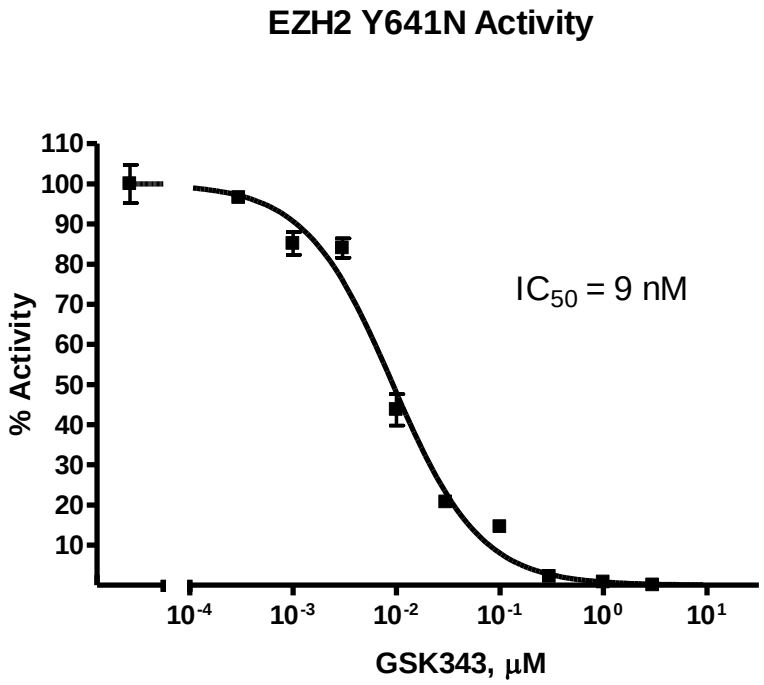
Example of Assay Results:



EZH2(Y641N) enzyme activity, measured using the *EZH2 (Y641N) Chemiluminescent Assay Kit*, BPS Bioscience # 52071. 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

Product Name	Catalog #	Size
EZH2 (Y641F)/EED/SUZ12/RbAp48/AEBP2	51017	20 µg
EZH2 (Y641C)/EED/SUZ12/RbAp48/AEBP2	51029	20 µg
EZH2 (Y641N)/EED/SUZ12/RbAp48/AEBP2	51028	20 µg
EZH2 (Y641S)/EED/SUZ12/RbAp48/AEBP2	51013	20 µg
EZH2 (Y641H)/EED/SUZ12/RbAp48/AEBP2	51011	20 µg
EZH2/EED/SUZ12/RbAp48/AEBP2	51004	50 µg
EZH2 Chemiluminescent Assay Kit	52009L	96 rxns.

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EZH2 (Y641F) Chemiluminescent Assay Kit	52075	96 rxns.

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	EZH2 (Y641N) Complex has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh EZH2 (Y641N) Complex, BPS Bioscience #51028. 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. See section on "Reading Chemiluminescence" above.
	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	Be sure to include blocking steps after wash steps. 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 (Y641N) Complex, BPS #51028 to create a standard curve.