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Data Sheet

SMYD2 Chemiluminescent Assay Kit

Catalog # w61042
Size: 96 reactions

DESCRIPTION: The *SMYD2 Chemiluminescent Assay Kit* is designed to measure SMYD2 activity for screening and profiling applications. The *SMYD2 Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well plate precoated with histone H3 substrate, antibody against methylated lysine residue of Histone H3, a secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified SMYD2 enzyme for 96 enzyme reactions. The key to the *SMYD2 Chemiluminescent Assay Kit* is a highly specific antibody that recognizes a methylated residue of Histone H3. With this kit, only three simple steps are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and the SMYD2 methyltransferase enzyme. 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	
w61025	SMYD2 human enzyme	80 µg	-80℃	***(Avoid freeze/ thaw cycles!)***
w62131	100 µM S-adenosylmethionine	500 µl	-80℃	
w62151P	Primary antibody 16	100 µl	-80℃	
w62142H	Secondary HRP-labeled antibody 2	10 µl	-80℃	
w62204	4x HMT assay buffer 7	3 ml	-20℃	
w62111	Blocking buffer	50 ml	+4℃	
	HRP chemiluminescent substrate A (translucent bottle)	6 ml	+4℃	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4℃	
	White microplate precoated with histone substrate	1	+4℃	

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

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt

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

REFERENCE: Brown MA, *et al. Mol. Cancer* 2006; **5**:26.

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 strip 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 contents of the tube. Aliquot **S-adenosylmethionine** into single use aliquots. Store remaining **S-adenosylmethionine** in aliquots at -80°C immediately. *Note: **S-adenosylmethionine** is sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

3) Prepare the master mixture: N wells × (7.5 µl **4x HMT assay buffer 7** + 5 µl 100 µM **S-adenosylmethionine** + 12.5 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 7** + 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) Prepare **1x HMT assay buffer 7** by diluting one part **4x HMT assay buffer 7** with three parts distilled water. Prepare only enough **1x HMT assay buffer 7** required for the assay. Add 20 µl of **1x HMT buffer 7** to the well designated "Blank".

7) Thaw **SMYD2 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **SMYD2 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **SMYD2 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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8) Dilute **SMYD2 enzyme** in **1x HMT assay buffer** to 40 ng/µl (800 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

	Blank	Substrate Control	Positive Control	Test Inhibitor
4x HMT assay buffer 7	7.5 µl	7.5 µl	7.5 µl	7.5 µl
100 µM S-adenosylmethionine	5 µl	–	5 µl	5 µl
H_2O	12.5 µl	17.5 µl	12.5 µl	12.5 µl
Test Inhibitor/Activator	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1x HMT assay buffer 7	20 µl	–	–	–
SMYD2 (40 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

9) Initiate reaction by adding 20 µl of diluted **SMYD2** (prepared as described above) to the wells labeled “Test Inhibitor”, “Positive Control”, and “Substrate Control”. Incubate overnight at room temperature on a rotating platform. Seal the wells if necessary.

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

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

Step 2:

1) Dilute **Primary antibody 16** 100-fold with **Blocking buffer**.

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

3) Wash plate three times with 200 µl TBST buffer. Add 100 µl of **Blocking buffer** to every well. Shake on a rotating platform for 10 min. Remove supernatant by blotting onto clean paper towels.

Step 3:

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

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

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3) Wash plate three times with TBST buffer. Add 100 µl of **Blocking buffer** to every well. Shake on a rotating platform for 10 min. Remove supernatant by blotting onto clean paper towels.

4) Just before use, on ice, mix 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent 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 reader 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 wavelength 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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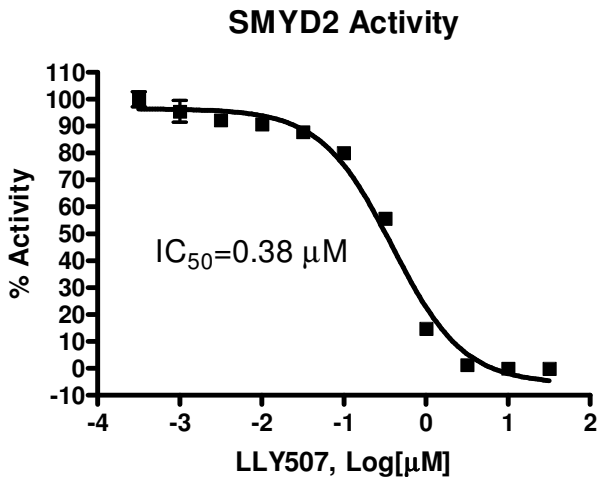
Example of Assay Results:



Inhibition of SMYD2 activity by LLY507, measured using the *SMYD2 Chemiluminescent Assay Kit*, West Bioscience Catalog #w61042. Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com.*

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	SMYD2 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (SMYD2, West Bioscience #w61025). Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for antibody incubation. Avoid freeze/thaw cycles of antibody.
	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 strip 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 enzyme (SMYD2, West Bioscience #w61025) to create a standard curve.