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Data Sheet

SMYD3 Homogeneous Assay Kit

Catalog # 52063
Size: 384 reactions

DESCRIPTION: The *SMYD3 Homogeneous Assay Kit* is designed to measure SMYD3 activity for screening and profiling applications. SMYD3 is a histone methyltransferase that promotes di- and trimethylation of lysine4 of histone H3. It also exhibits methylation activity toward a lysine residue of MAPK/ERK Kinase Kinase 2 (MAP3K2). The *SMYD3 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with GST-tagged SMYD3 substrate, primary antibody, methylation assay buffer, and purified SMYD3 for 384 enzyme reactions. The key to the *SMYD3 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 SMYD3 enzyme is incubated with the substrate for three hours. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51110	SMYD3/HSP90	20 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120	100 µM S-adenosylmethionine	2 x 250 µl	-80°C	
52140O	Primary antibody 15	5 µl	-80°C	
	SMYD3 substrate	200 µl	-80°C	
	4x SMYD3 assay buffer*	3 x 1 ml	-20°C	
	4x Detection buffer 3	2 ml	-20°C	

*Add 5 µl of 0.5M DTT to each 1 ml tube before use.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA® anti-rabbit IgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
AlphaScreen® Glutathione donor beads, 5 mg/ml (PerkinElmer #6765300)
Optiplate-384 (PerkinElmer #6007290)

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.

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REFERENCES:
1. Dillon, S.C., *et al.* 2005. *Genome Biology* **6:**227.
2. Silva, F.P., *et al.* 2008. *Oncogene* **27:**2686.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Add 5 µl of 0.5M DTT to each 1 ml tube before use. Prepare the master mixture: N wells x (2.0 µl **4x SMYD3 Assay buffer** + 1.0 µl **S-adenosylmethionine** (100 µM) + 0.5 µl **SMYD3 substrate** + 1.5 µl water). Add 5 µl to wells designated "Positive Control", "Test Sample", and "Blank". To wells labeled "Substrate Control", add 2 µl **4x SMYD3 Assay buffer** + 0.5 µl **SMYD3 substrate** + 2.5 µl water.

2) Add 3 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 3 µl of the same solution without inhibitor (Inhibitor buffer).

3) Prepare **1x SMYD3 Assay buffer** by adding 1 part of **4x SMYD3 Assay buffer** to 3 parts water (v/v). Prepare only enough for the assay; store any remaining **4x SMYD3 Assay buffer** at -20˚C.

4) Thaw **SMYD3** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **SMYD3** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: SMYD3 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme*. *Depending on the nature of inhibitor, pre-incubation with the enzyme may be necessary.*

5) To the wells designated as "Blank", add 2 µl of **1x SMYD3 Assay buffer**.

6) Dilute **SMYD3** in **1x SMYD3 Assay buffer** at 13-25 ng/µl (26-50 ng/2 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

7) Initiate reaction by adding 2 µl of diluted **SMYD3** enzyme to the wells designated "Positive Control", "Substrate Control", and "Test Sample". Incubate at room temperature for 3-4 hours.

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	Positive Control	Test Sample	Substrate Control	Blank
4x SMYD3 Assay buffer	2 µl	2 µl	2 µl	2 µl
100 µM S-adenosylmethionine	1 µl	1 µl	–	1 µl
SMYD3 substrate	0.5 µl	0.5 µl	0.5 µl	0.5 µl
H_2O	1.5 µl	1.5 µl	2.5 µl	1.5 µl
Test Inhibitor/Activator	–	3 µl	–	–
Inhibitor Buffer (no inhibitor)	3 µl	–	3 µl	3 µl
1x SMYD3 Assay buffer	–	–	–	2 µl
SMYD3 (13-25 ng/µl)	2 µl	2 µl	2 µl	–
Total	**10 µl**	**10 µl**	**10 µl**	**10 l**

Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:

1) Prepare **1x Detection buffer 3** by adding 1 part **4x Detection buffer 3** to 3 parts water (v/v). Prepare only enough for the assay; store any remaining **4x Detection buffer 3** at -20˚C.

2) Dilute anti-Rabbit Acceptor beads 1:250-fold with **1x Detection buffer 3**. Add 5 µl per well. Shake plate briefly.

3) Dilute **Primary antibody 15** 400-fold with **1x Detection buffer 3**. Add 5 µl per well. Shake plate. Incubate 30 min at room temperature.
(Alternatively, dilute anti-Rabbit Acceptor beads (1:500) and Primary antibody 4 (1:800) with 1x Detection buffer 3 in one step. Add 10 µL of acceptor beads/antibody mixture per well.)

Step 3:

1) Dilute Glutathione donor beads 125-fold with **1x Detection buffer 3**. Add 10 µl per well. Incubate for 30-60 min. at room temperature.
2) Read Alpha-counts.

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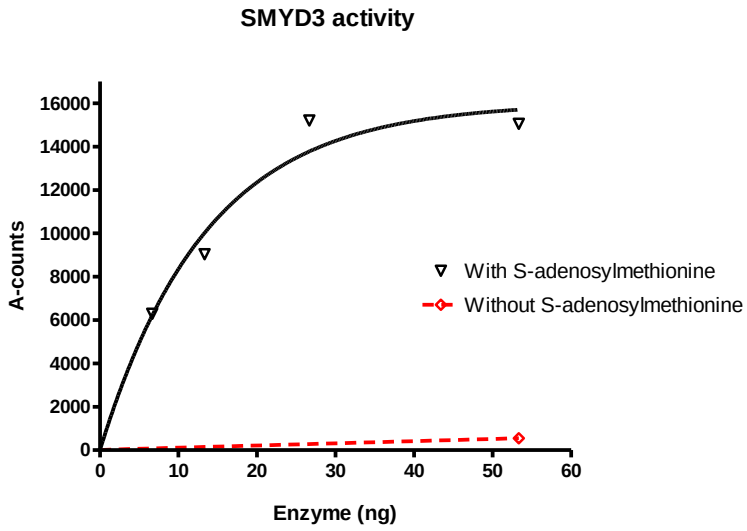
Example of Assay Results:



SMYD3 enzyme activity, measured using the *SMYD3 Homogeneous Assay Kit*, BPS Bioscience Cat. #.... *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

SMYD3 (full length), FLAG-tag	#51031	20 µg
SMYD3 (35-end), GST-tag	#51015	20 µg
SMYD2 (KMT3C), FLAG-tag	#51014	20 µg
SMYD2 (KMT3C) Chemiluminescent Assay Kit	#52055	96 reactions

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