Description

The SMYD3 Homogeneous Assay Kit is designed to measure SMYD3 (SET and MYND domain containing protein 3) activity for screening and profiling applications. The SMYD3 Homogeneous Assay Kit comes in a convenient 384-well AlphaLISA® format, with enough purified recombinant SMYD3/HSP90 complex, GST-tagged SMYD3 substrate, primary antibody and methylation assay buffer for 384 enzyme reactions.

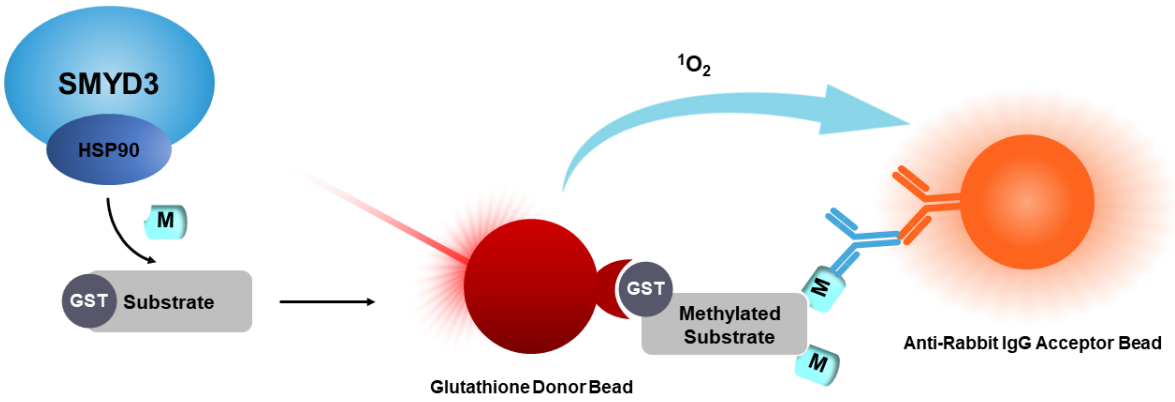

Figure 1: *SMYD3 Homogenous Assay Kit schematic.*
A sample containing SMYD3/HSP90 complex is incubated with a substrate for four hours. This is followed by the addition of acceptor beads and primary antibody, and finally donor beads. Alpha-counts are then counted. Alpha-counts are proportional to SMYD3 methylase activity.

Background

SMYD3, also known as SET (suppressor of variegation, enhancer of Zeste, Thithorax) and MYND (myeloid-Nervy-DEAF-1) domain-containing protein 3 is a lysine methyltransferase family member. It is involved in methylation of H3K4 and H4K5, but also of non-histone substrates such as VEGFR1 (vascular endothelial growth factor receptor 1), MAP3K2 (MAP3 kinase 2), AKT1 (protein kinase B) and HER2 (human epidermal growth factor receptor 2). In adults it is found in platelets, testis, $CD8^+$ T-cells, and at very low levels in the heart, bladder, prostate, spinal cord and retina. It is overexpressed in several cancer types, such as liver and breast cancer, and plays a role in disease progression via its substrates. For instance, SMYD3 activates AKT1 by methylation on lysine 14, resulting in cell growth, survival and neovascularization. The low or absence expression of SMYD3 in normal tissues makes it a promising cancer therapy candidate. Several inhibitors are undergoing clinical trials, but further studies are necessary. A deep understanding of the role of SMYD3 and identification of potent specific inhibitors will advance the cancer therapy field.

Application(s)

Study enzyme kinetics and screen small molecule inhibitors in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
51110	SMYD3/HSP90*	2 x 50 µg	-80°C
52120	100 µM S-adenosylmethionine	2 x 250 µl	-80°C
52140O3	Primary Antibody 15-3	20 µl	-80°C
	SMYD3 Substrate	2 x 200 µl	-80°C
	4x SMYD3 Assay Buffer	3 x 1 ml	-20°C
	4x Detection Buffer 3D	2 ml	-20°C
	0.5 mM DTT	200 µl	-20°C
	Plate Sealer		Room Temp.

**The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

Name	Catalog #
AlphaLISA® anti-rabbit IgG acceptor beads	Perkin Elmer #AL104C
AlphaScreen® Glutathione-conjugated donor beads	Perkin Elmer #6765300
Optiplate - 384	Perkin Elmer #6007290
AlphaScreen® microplate reader	

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The SMYD3 Homogenous Assay Kit is compatible with up to 1% final DMSO concentration.
- Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (λ=520-620 nm), such as Trypan Blue.
- Avoid the use of 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. Media like MEM, which lacks these components, does not affect AlphaScreen assays.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control", "Substrate Control" and "Test inhibitor".
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- Depending on the nature of the inhibitor, pre-incubation of the enzyme with the inhibitor may be necessary.

Step 1:

1. Thaw **0.5 mM DTT**, **100 µM S-adenosylmethionine**, **4x SMYD3 Assay Buffer** and **SMYD3 Substrate**.

2. Add 5 µl of 0.5 M DTT 50 to 1 ml of 4x SMYD3 Assay Buffer. This makes 4x SMYD3 Assay Buffer with DTT.

3. Prepare a Master Mix (5 µl/well): N wells x (2 µl of 4x SMYD3 Assay Buffer with DTT + 1 µl of 100 µM S-adenosylmethionine + 1 µl of SMYD3 Substrate + 1 µl of distilled water).

4. Add 5 µl of Master Mix to all wells, except the "Substrate Control" wells.

5. Prepare a Deficient Master Mix (5 µl/well): N wells x (2 µl of 4x SMYD3 Assay Buffer with DTT + 1 µl of 100 µM S-adenosylmethionine + 2 µl of distilled water).

6. Add 5 µl of Deficient Master Mix to the "Substrate Control" wells.

7. Prepare the Test Inhibitor (3 µl/well): for a titration prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 15 µl.

   7.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water, 5-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use distilled water (Diluent Solution).

   OR

   7.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 20-fold in distilled water to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

   Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in distilled water to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 5% DMSO in water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

8. Add 3 µl of inhibitor solution to each well designated "Test Inhibitor".
9. Add 3 µl of Diluent Solution to the "Blank", "Positive Control" and "Substrate Control" wells.
10. Dilute 4x SMYD3 Assay Buffer with DTT 4-fold with distilled water. This makes 1x SMYD3 Assay Buffer.

    Note: Prepare only enough to perform the assay. Store remaining 4x SMYD3 Assay Buffer at -20°C.
11. Thaw SMYD3 on ice. Briefly spin the tube containing enzyme to recover the full content of the tube.
12. Dilute SMYD3 to 75-100 ng/µl with 1x SMYD3 Assay Buffer (2 µl/well).
13. Initiate the reaction by adding 2 µl of diluted SMYD3 to the wells labeled "Positive Control", "Substrate Control" and "Test Inhibitor".
14. Add 2 µl of 1x SMYD3 Assay Buffer to the "Blank" wells.
15. Seal the wells with the plate sealer.
16. Incubate at 30°C for 4 hours with slow agitation.

Protect your samples from direct exposure to light for step 2 and 3. Photobleaching will occur!

Component	Test Inhibitor	Blank	Positive Control	Substrate Control
Master Mix	5 µl	5 µl	5 µl	-
Deficient Master Mix	-	-	-	5 µl
Test Inhibitor	3 µl	-	–	-
Diluent Solution	–	3 µl	3 µl	3 µl
1x SMYD3 Assay Buffer	-	2 µl	-	-
Diluted SMYD3 (75-100 ng/µl)	2 µl	-	2 µl	2 µl
Total	**15 µl**	**15 µl**	**15 µl**	

Step 2:

1. Dilute 4-fold the 4X Detection Buffer 3D with distilled water. This makes 1X Detection Buffer 3D.

   Note: Prepare only enough to perform the assay. Store remaining 4x Detection Buffer 3D at -20°C.
2. Dilute anti-rabbit acceptor beads diluted 250-fold with 1X Detection Buffer 3D (5 µl of mix/well). Mix well.
3. Add 5 µl of diluted anti-rabbit acceptor beads each well.
4. Briefly agitate the plate.
5. Dilute 100-fold the Primary Antibody 15-3 with 1x Detection Buffer 3D (5 µl of mix/well). Mix well.

6. Add 5 µl of diluted Primary Antibody 15-3 to each well.
7. Briefly agitate the plate.
8. Incubate 30 minutes at Room Temperature (RT).

Step 3:

1. Dilute Glutathione donor beads 125-fold with 1x Detection Buffer 3D (10 µl/well).
2. Add 10 µl of diluted Glutathione donor beads to each well.
3. Incubate 10-15 minutes at RT.
4. Read Alpha-Counts.
5. The "Blank" control might be important to determine the background A-screen counts in the assay. The blank value should be subtracted from all other values.

Example Results

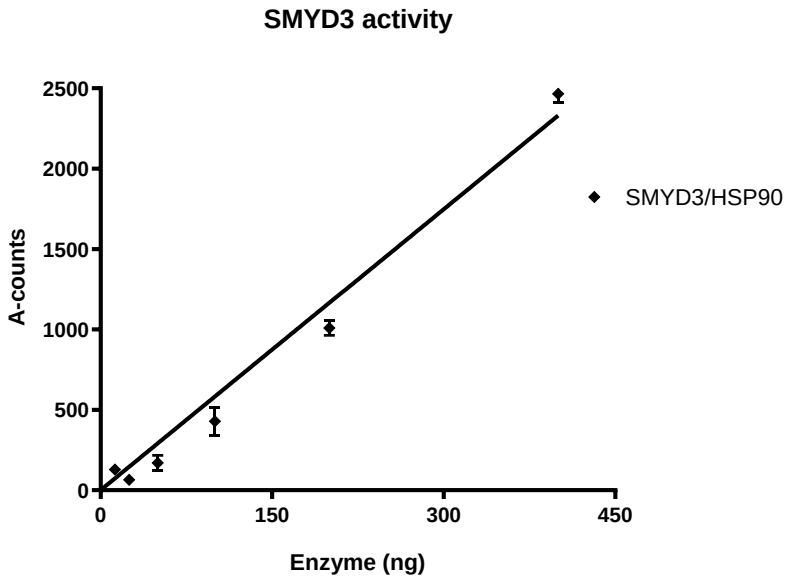

Figure 2: SMYD3 activity.
SMYD3 activity was measured with different concentrations of SMYD3/HSP90 complex.

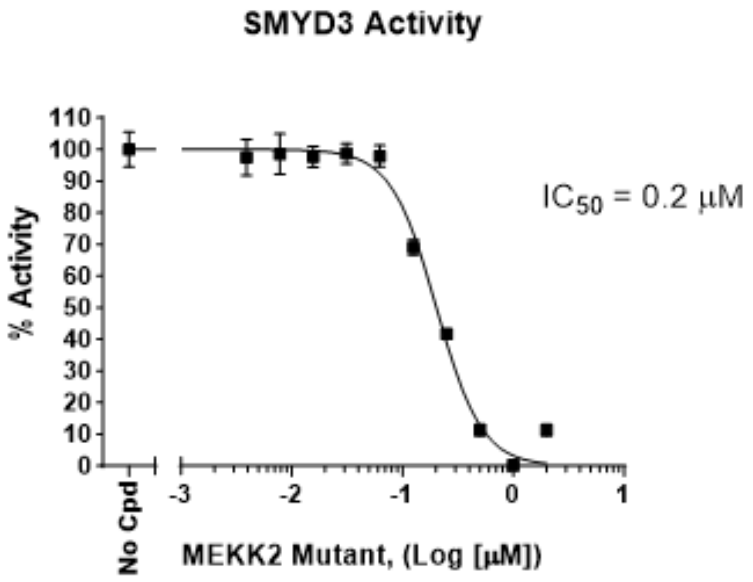

Figure 3: SMYD3 activity is inhibited by MEKK2 Mutant.
SMYD3 were incubated with increasing concentrations of MEKK2 Mutant.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Dillon S.C., *et al.* 2005 *Genome Biology* 6:227.
Silva F.P., *et al.* 2008 *Oncogene* 27:2686.
Bernard B., *et al.*, 2021 *Clin Epigenetics* 13:45.

Related Products

Products	*Catalog #*	*Size*
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

Version 010524