Description

The MLL4 (KMT2B) Complex Chemiluminescent Assay Kit is designed to measure MLL4 (mixed lineage leukemia protein-4, also known as KMT2B) /WARD complex activity for screening and profiling applications. The MLL4/ WARD complex contains MLL4, WDR5 (WD40 repeat-containing protein 5), Ash2L (Absent, small, homeotic disks-2-like), RbBP5 (retinoblastoma-binding protein 5) and DPY30 (DumPY protein 30). The MLL4 (KMT2B) Complex Chemiluminescent Assay Kit comes in a convenient format, with 8-well strips pre-coated with histone H3 peptide substrate, primary antibody, a secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and enough purified MLL4/WARD complex for 100 enzyme reactions.

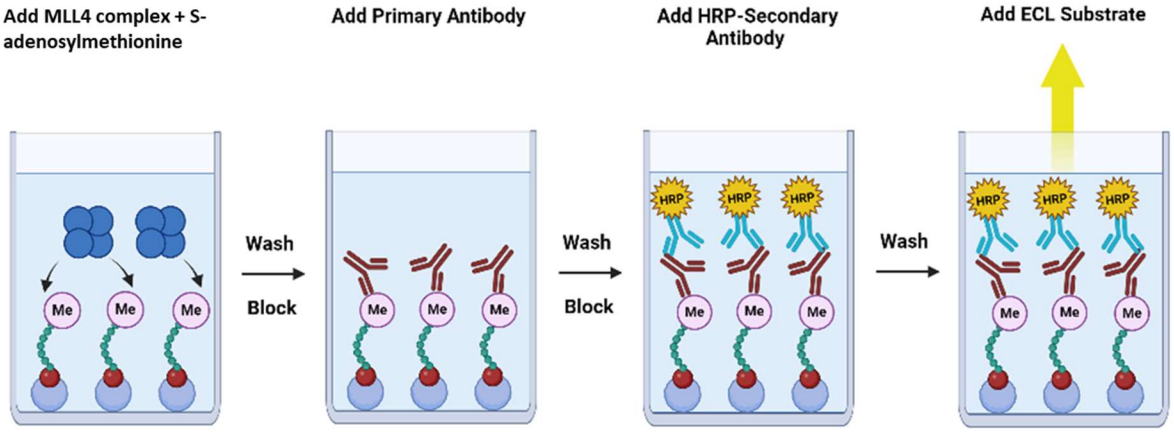

Figure 1: Illustration of the assay principle.

First, the MLL4 enzyme complex and S-adenosylmethionine are added to pre-coated with histone H3 peptide substrate plate and incubated with or without a test compound. Next, the primary antibody is added. Finally, an HRP-labeled secondary antibody is added, followed by the addition of the HRP substrate to produce chemiluminescence that can be measured using a chemiluminescence reader. Chemiluminescence signal is proportional to the enzymatic activity of the MLL4/WARD complex.

Background

The MLL4 (Mixed Lineage Leukemia-4) protein, also known as KMT2B, belongs to the SET1/MLL family which consists of six (MLL1-4/KMT2A-2D, SET1A/KMT2F, and SET1B/KMT2G) major methyltransferases in mammals. MLL4 is a histone-H3 lysine-4 (H3K4) methyltransferase that promotes H3K4 mono-/di-/tri-methylation, a conserved trait of euchromatin associated with transcriptional activation. MLL4 is a critical player for memory formation. MLL4 forms a complex with RbBP5 (retinoblastoma-binding protein 5), ASH2L (Absent, small, homeotic disks-2-like), WDR5 (WD40 repeat-containing protein 5), and DPY30 (DumPY protein 30) to catalyze methylation of H3K4. WDR5 has two protein interaction sites: the WDR5-interacting (WIN) binding site and the WDR5-binding-motif (WBM) site. MLL4 forms the complex via the WIN binding site, while RbBP5 is bound to the WBM site, which is also the site for MYC oncoproteins interaction. WDR5 represents a therapeutically exploitable target for cancer inhibition as it plays a crucial role in MLL4 complex assembly and disassembly. The addition of inhibitors that competitively bind to WIN or WBM sites has been shown to disrupt MLL activity as well as to displace MYC from chromatin and therefore disabling its tumorigenic function.

Applications

Screen or titer small molecule inhibitors in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100324	MLL4/WDR5/Ash2L/RbBP5/DPY30 -WARD Complex (His-Tagged)*	5 µg	-80°C
52120	20 µM S-adenosylmethionine	2 x 250 µl	-80°C
52140Z	Primary Antibody 26	10 µl	-80°C
52160	4x HMT Assay Buffer 1	3 ml	-20°C
52131H	Secondary HRP-Labeled Antibody 2	10 µl	-80°C
52100	Blocking Buffer 4	50 ml	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
	0.5 M DTT	200 µl	-20°C
	96-well plate pre-coated with histone substrate	1 plate	+4°C

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

Materials Required but Not Supplied

- TBST Buffer (1 x TBS, pH 8.0, containing 0.05% Tween-20)
- Microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Stability

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 MLL4 (KMT2B) Complex Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

- We recommend using MM-102 (TFA), SAH (#52031) or Chaetocin (#27221) as an internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1: MLL4 Methylation/Methyltransferase Activity

1. Rehydrate each pre-coated well with 200 µl of TBST Buffer.
2. Incubate for five minutes at Room Temperature (RT).
3. Tap the plate onto clean paper towel to remove the liquid.
4. Add 125 µl of 0.5 M DTT to 4x HMT Assay Buffer 1.
5. Prepare 1x HMT Assay Buffer 1 by diluting 4-fold the 4x HMT Assay Buffer 1 containing DTT with distilled water.
6. Add 15 µl of 1x HMT Assay Buffer 1 to all wells.
7. Thaw **MLL4 complex**, on ice. Briefly spin the tube to recover the full content.
8. Dilute MLL4 enzyme complex to 2.5 ng/µl in **1x HMT Assay Buffer 1** (20 µl/well).
9. Add 20 µl of diluted MLL4 complex to all wells, except the "Blank" wells.
10. Add 20 µl of 1x HMT Assay Buffer 1 to the "Blank" wells.
11. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

    11.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in 1x HMT Assay Buffer 1.

    For the positive and negative controls, use 1x HMT Assay Buffer 1 (Diluent Solution).

    OR

    11.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x HMT Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

    Using 1x HMT Assay Buffer 1 containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

For positive and negative controls, prepare 10% DMSO in 1x HMT Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

12. Add 5 µl of inhibitor solution to each well designated "Test Inhibitor".
13. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
14. Preincubate the inhibitor with the diluted MLL4 for 30 minutes at RT with gentle agitation.
15. Thaw **20 µM S-adenosylmethionine** on ice. Briefly spin the tube to recover the full content.

    Note: Aliquot S-adenosylmethionine into single use aliquots (minimum volume of 5 µl/aliquot). Store remaining S-adenosylmethionine in aliquots at -80°C immediately.

16. Dilute 20 µM S-adenosylmethionine 2-fold with 1x HMT Assay Buffer 1 (10 µl/well).
17. Add 10 µl of diluted S-adenosylmethionine to all wells.
18. Incubate at RT for 1 hour with gentle agitation.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
1x HMT Assay Buffer 1	35 µl	15 µl	15 µl
Diluted MLL4 (2.5 ng/µl)	-	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
30 minutes at Room Temperature			
Diluted S-adenosylmethionine (10 µM)	10 µl	10 µl	10 µl
Total	**50 µl**	**50 µl**	**50 µl**

19. Tap the plate onto clean paper towel to remove the liquid.
20. Wash the plate three times with 200 µl of TBST Buffer per well.
21. Tap the plate onto clean paper towel to remove the liquid.
22. Add 100 µl of **Blocking Buffer 4** to every well.
23. Incubate for 10 minutes at RT.
24. Tap the plate onto clean paper towel to remove the liquid.

Step 2: Detection

1. Dilute 1000-fold the **Primary Antibody 26** with **Blocking Buffer 4** (100 µl/well).
2. Add 100 µl of diluted Primary Antibody 26 per well.
3. Incubate at RT for 1 hour with gentle agitation.
4. Tap the plate onto clean paper towel to remove the liquid.
5. Wash the plate three times with 200 µl of TBST Buffer per well.
6. Tap the plate onto clean paper towel to remove the liquid.
7. Add 100 µl of **Blocking Buffer 4** to every well and incubate for 10 minutes.
8. Tap the plate onto clean paper towel to remove the liquid.
9. Dilute 1,000-fold the **Secondary HRP-Labeled Antibody 2** with **Blocking Buffer 4** (100 µl/well).
10. Add 100 µl of diluted Secondary HRP-Labeled Antibody 2 per well.
11. Incubate at RT for 30 minutes with gentle agitation.
12. Tap the plate onto clean paper towel to remove the liquid.
13. Wash the plate three times with 200 µl of TBST Buffer per well.
14. Just before use, mix on ice 50 µl **ELISA ECL Substrate A** and 50 µl **ELISA ECL Substrate B** (100 µl of mix/well).
15. Add 100 µl of mix per well.
16. Immediately read sample in a microtiter-plate capable of reading chemiluminescence.
17. The Blank value should be subtracted from all other values.

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 you are using your plate reader in a LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Make sure you don't have filter when emit the light (Synergy 2 BioTek: use "hole" position on filter wheel). Optics position – Top. Read type: endpoint. Sensitivity may be adjusted based on luminescence of a control without enzyme (typically we set this value as 100 when using Synergy 2 plate reader).

Example Results

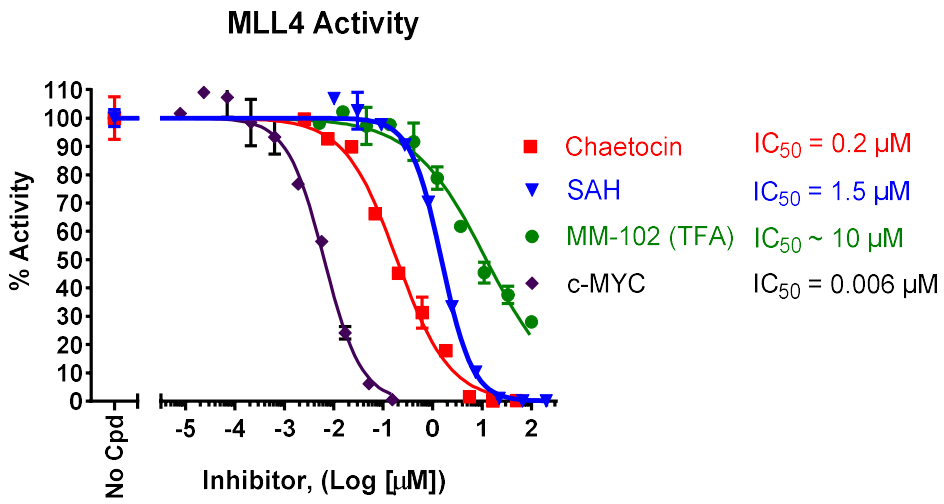

Figure 2: Inhibition of MLL4 activity by various inhibitors and by c-MYC protein.
MLL4 activity was measured in the presence of increasing concentrations of Chaetocin (BPS Bioscience #27221), SAH (BPS Bioscience #52031), MM-102 (TFA) (MedChemExpress #HY-12220A) and c-MYC protein (BPS Bioscience #40453). Results are expressed as percent of control (MLL4 activity in the absence of inhibitor, set at 100%).

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
Wang L., *et al.*, 2021 *Biochem Soc Trans* 49(6):1041-1054.

Related Products

Products	*Catalog #*	*Size*
MLL1/WDR5/Ash2L/RbBP5/DPY30 (MLL1/WARD Complex), His-Tag	51022	50 µg
MLL3/WDR5/Ash2L/RbBP5/DPY30 Complex Recombinant	100323	50 µg
WDR5, FLAG-Tag Recombinant	51111	100 µg
Anti-WDR5 Polyclonal Antibody	25321	100 µl
MLL1 Complex Chemiluminescent Assay Kit	53008	96 reactions
MLL3 Complex Chemiluminescent Assay Kit	79758	96 reactions

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