Description

The MLL4 (KMT2B): WDR5 Binding Chemiluminescent Assay Kit is an ELISA-based assay designed to measure the binding between MLL4 (mixed lineage leukemia protein-4, also known as KMT2B) and WDR5 (WD40 repeat-containing protein 5) for screening and profiling applications. The MLL4 (KMT2B): WDR5 Binding Chemiluminescent Assay comes with enough purified MLL4 (amino acids 2490-2715) and WDR5 proteins, primary and secondary antibody, assay buffer, and detection reagent for 100 enzyme reactions.

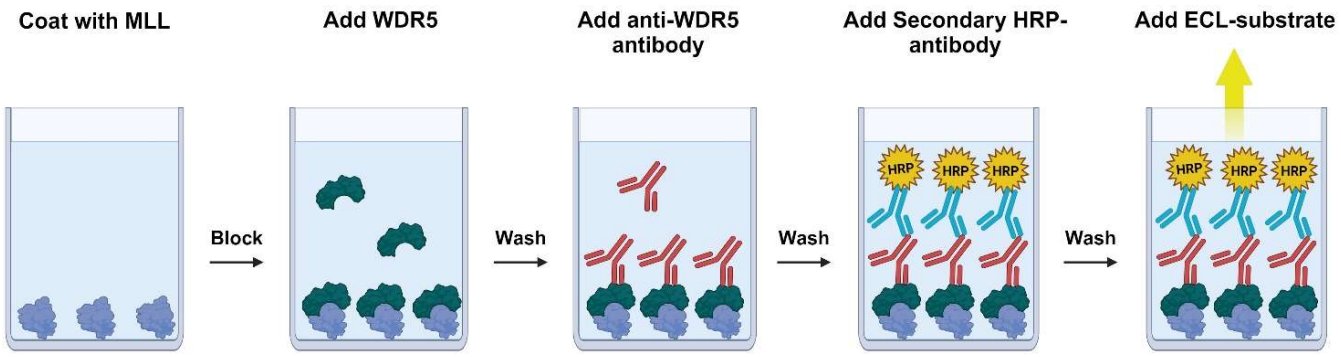

Figure 1. MLL4 (KMT2B): WDR5 Binding Chemiluminescent Assay Kit schematic.
A 96-well plate is coated with MLL4 protein. After coating and blocking, WDR5 is added in an optimized assay buffer. Next, unbound WDR5 is washed away, and the plate is incubated with a primary antibody followed by a secondary HRP-conjugated antibody. Finally, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the efficacy of WDR5 binding to MLL4 (KMT2B).

Background

The Mixed Lineage Leukemia-4 (MLL4, also known as KMT2B) protein 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 in 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 represents a therapeutically exploitable target for cancer treatment as it plays a crucial role in MLL1 complex assembly and disassembly. WDR5 has two protein interaction sites: the WDR5-interacting (WIN) binding site and the WDR5-binding-motif (WBM) site. MLL1-4 forms the complex via WIN binding site, while RbBP5 is bound to WBM site, which is also the site for MYC oncoproteins interaction. 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

Study complex formation and screen compounds that block the binding of MLL4 to WDR5 for drug discovery and high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
11344	MLL4, His-Tag*	2.5 µg	-80°C
79187	WDR5, FLAG-Tag*	2.5 µg	-80°C
	3x PL-02 Assay Buffer	50 µl	-80°C
	Primary Antibody AB31	6 µl	-80°C
52130H	Secondary HRP-Labeled Antibody 1	10 µl	-80°C
	Blocking Buffer 8	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
79837	96-well module plate	1	Room Temp

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

Materials Required but Not Supplied

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

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 MLL4 (KMT2B): WDR5 Binding 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 as 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: Coat 96-well module

1. Thaw MLL4 on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute MLL4 protein to 0.5 ng/µl with TBS (50 µl/well).
3. Add 50 µl of diluted MLL4 to every well except "Blank" wells.
4. Add 50 µl of Blocking Buffer 8 to "Blank" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times using 200 µl of TBST Buffer per well.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Block the wells by adding 200 µl of Blocking Buffer 8 to every well.
9. Incubate at Room Temperature (RT) for at least 90 minutes.
10. Wash the plate three times using 200 µl of TBST Buffer per well.
11. Tap the plate onto clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Prepare 1x Assay Buffer by diluting 3x PL-02 Assay Buffer 3-fold with distilled water.
2. Wash the plate using 100 µl of 1x Assay Buffer per well.
3. Tap the plate onto clean paper towel to remove the liquid.
4. Add 20 µl of 1x Assay Buffer to every well.
5. 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.

   5.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using 1x Assay Buffer.

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

   OR

5.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x Assay Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

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

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

6. Add 5 µl of Test Inhibitor to each well labeled as "Test Inhibitor".
7. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
8. Thaw WDR5 on ice. Briefly spin the tube containing the protein to recover its full content.
9. Dilute WDR5 to 1 ng/µl with 1x Assay Buffer (25 µl/well).
10. Add 25 µl of diluted WDR5 to all wells.
11. Incubate at RT for 1 hour.

	Blank (non-coated wells)	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted WDR5 (1 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

12. Wash the plate three times with 200 µl of TBST Buffer per well and tap the plate onto clean paper towel.

Step 3: Detection

1. Dilute 1000-fold the Primary Antibody AB31 with Blocking Buffer 8 (50 µl/well).
2. Add 50 µl of diluted Primary Antibody AB31 to every well.
3. Incubate for 60 minutes at RT.
4. Wash the plate three times with 200 µl of TBST Buffer per well and tap the plate onto clean paper towel.

5. Dilute 1000-fold the Secondary HRP- Antibody 1 with Blocking Buffer 8 (50 µl/well).

6. Add 50 µl of diluted Secondary antibody to every well.

7. Incubate for 30-45 minutes at RT.

8. Wash the plate three times with 200 µl of TBST Buffer per well and tap the plate onto clean paper towel.

9. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/ well).

10. Add 100 µl of mix to every well.

11. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

12. 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 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).

Example Results

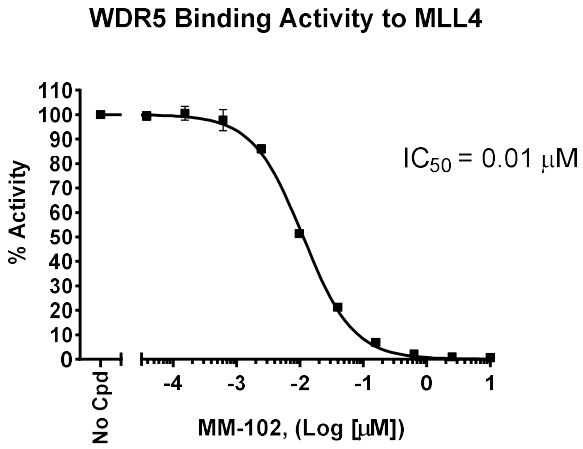

Figure 2: Inhibition of MLL4 – WDR5 binding by MM-102.
WDR5 was incubated with increasing concentrations of MM-102 in an MLL4 coated plate. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding activity in which the condition without MM-102 is set to 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 (KMT2A): WDR5 Binding Chemiluminescent Assay Kit	82502	96 reactions
WDR5, His-tag Recombinant	71200	100 µg
Anti-WDR5 polyclonal antibody	25321	100 µl
MLL4 (KMT2B): WARD Complex Chemiluminescent Assay Kit	79757	96 reactions
MLL3 (KMT2C): WARD Complex Chemiluminescent Assay Kit	79758	96 reactions
MLL1 (KMT2A): WARD Complex Chemiluminescent Assay Kit	53008	96 reactions

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