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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The c-Myc: WDR5 [Biotinylated] Binding Chemiluminescent Assay Kit is an ELISA designed to measure the binding between c-Myc and WDR5 (WD40 repeat domain 5) protein for screening and profiling applications. This kit comes with enough purified c-Myc (amino acids 2-454(end)) and biotinylated WDR5 (amino acids 2-334(end)), MLL1 (amino acids 3745-end), streptavidin-HRP, assay buffer, and detection reagent for 100 enzyme reactions.

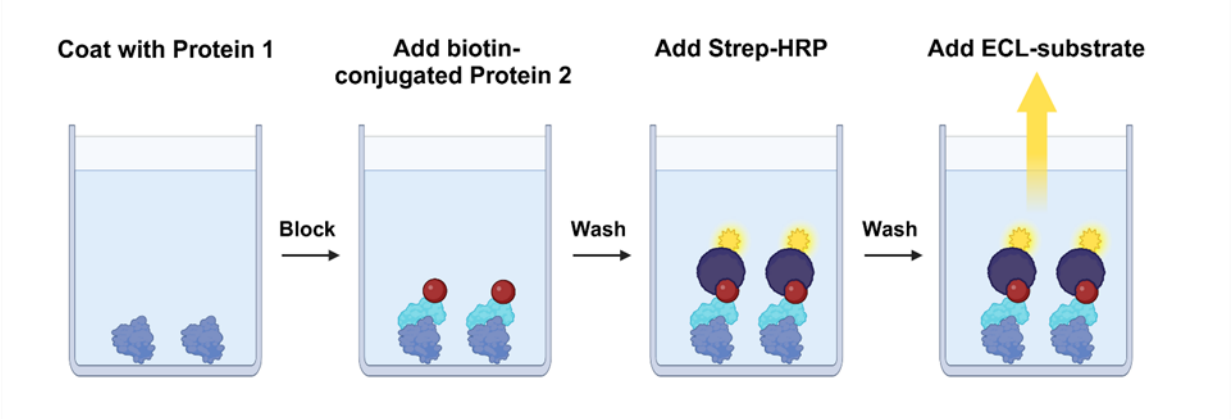

Figure 1. c-Myc: WDR5 [Biotinylated] Binding Chemiluminescent Assay Kit schematic.
A 96-well plate is coated with c-Myc protein. After coating and blocking, MLL1 and biotinylated WDR5 are added in an optimized assay buffer. Next, unbound biotinylated WDR5-MLL complexes are washed away, and the plate is incubated with streptavidin-HRP. After a final wash, 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 c-Myc.

Background

WDR5 (WD40 repeat-containing protein 5) is a protein that is part of the histone methyltransferase complex SET1/MLL (Protein Mixed Lineage Leukemia) which catalyzes histone 3 lysine 4 methylation (H3K4me). In addition to its direct role in gene regulation by impacting histone methylation, WDR5 can induce transcription of c-Myc induced genes, by bringing c-Myc to the appropriate regions of the chromosome. WDR5 has two protein interaction sites: the WDR5-interacting (WIN) binding site and the WDR5-binding-motif (WBM) site. MLL1 forms the complex via the WIN binding site, while RbBP5 is bound to WBM site, which is also the site for c-Myc oncoproteins interaction. WDR5 is found at high levels in certain cancers, as is c-Myc. The addition of inhibitors that competitively bind to WIN or WBM sites has been shown to disrupt MLL activity as well as displace c-Myc from chromatin and therefore disabling its tumorigenic function.

Applications

Study and screen compounds that inhibit the binding of c-Myc to WDR5 for drug discovery in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40453	c-Myc, His-tag*	5 µg	-80°C
80016	WDR5, His-Tag, Biotin-Labeled *	5 µg	-80°C
79186	MLL1, His-Tag*	5 µg	-80°C
82952	PP-03 Buffer	10 ml	-20°C
79728	Blocking Buffer 2	25 ml	+4°C
79742	Streptavidin HRP	10 µl	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

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

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline) Buffer
- PBST Buffer (1x PBS, 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

This 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 WM-586 (MedChem #HY-153728) 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.

- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Coat 96-well plate

Coat the plate one day prior to running your samples.

1. Thaw c-Myc on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute c-Myc protein to 1 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted c-Myc to every well, except "Blank" wells.
4. Add 100 µl of Blocking Buffer 2 to the "Blank" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times using 200 µl of PBST Buffer per well.
7. Tap the plate onto a clean paper towel to remove the liquid.
8. Block the wells by adding 200 µl of Blocking Buffer 2 to every well.
9. Incubate at Room Temperature (RT) for at least 90 minutes.
10. Wash the plate three times using 200 µl of PBST Buffer per well.
11. Tap the plate onto a clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Thaw MLL1 on ice. Briefly spin the tube containing the protein to recover its full content.

Note: Although MLL1 is not required for c-MYC binding to WDR5, its presence improves complex formation.

2. Dilute MLL1 to 2.5 ng/µl with PP-03 Buffer (20 µl/well).
3. Add 20 µl of diluted MLL1 to every well.
4. Prepare the Test Inhibitor/Blocker (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.

   4.1 If the Test Inhibitor/Blocker is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using PP-03 Buffer.

   For the positive and negative controls, use PP-03 Buffer (Diluent Solution).

OR

4.2 If the Test Inhibitor/Blocker 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 PP-03 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 PP-03 Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in PP-03 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%.

5. Add 5 µl of Test Inhibitor to each well labeled as "Test Inhibitor".
6. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
7. Thaw WDR5 on ice. Briefly spin the tube containing the protein to recover its full content.
8. Dilute WDR5 to 2 ng/µl with PP-03 Buffer (25 µl/well).
9. Add 25 µl of diluted WDR5 to all wells.
10. Incubate at RT for 1 hour.

	Blank (non-coated wells)	**Positive Control**	**Test Inhibitor**
Diluted MLL1(2.5 ng/µl)	20 µl	20 µl	20 µl
Test Inhibitor/Blocker	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted WDR5 (2 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

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

Step 3: Detection

1. Dilute 1000-fold the Streptavidin-HRP with Blocking Buffer 2 (50 µl/well).
2. Add 50 µl of diluted Streptavidin-HRP to every well.
3. Incubate for 1 hour at RT.

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

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

6. Add 100 µl of mix to every well.

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

8. 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 controls.

Example Results

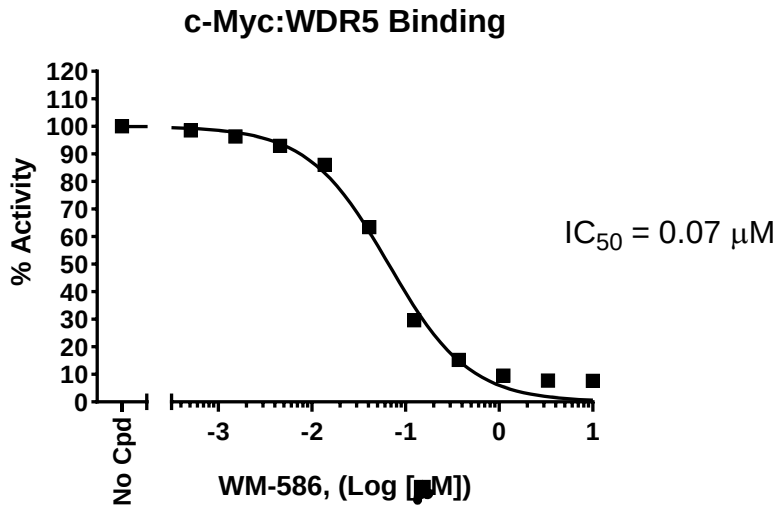

Figure 2: Inhibition of c-Myc- WDR5 binding by WM-586.
WDR5 was incubated with increasing concentrations of WM-586 (MedChem, #HY-153728) in a c-Myc coated plate and in the presence of MLL1. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding activity in which the condition without WM-586 is set to 100%.

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

References

Thomas L., *et al.*, 2020 *Molecular and Cellular Oncology* 7(2): 1709388.

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
MLL1 (KMT2A): WDR5 Binding Chemiluminescent Assay Kit	82502	96 reactions
MML4 (KMT2B): WDR5 Binding Chemiluminescent Assay Kit	82503	96 reactions
MLL3 Complex Chemiluminescence Assay Kit	79758	96 reactions
MLL1 Complex Chemiluminescent Assay Kit	53008	96 reactions
Anti-WDR5 polyclonal antibody	25321	100 µl

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