Description

The BLM ATPase Activity Assay Kit is designed for screening and profiling of BLM (Bloom syndrome protein) antagonists/inhibitors by monitoring their effect on the conversion of ATP to ADP using ADP-Glo™ as a detection reagent. ATP conversion to ADP occurs during DNA unwinding by the ATP dependent helicase domain of BLM. BLM ATPase Activity Assay Kit comes in a convenient 96-well format, with enough purified recombinant BLM (amino acids 630-1300), ATP, WRN substrate, assay buffer and additives for 100 reactions.

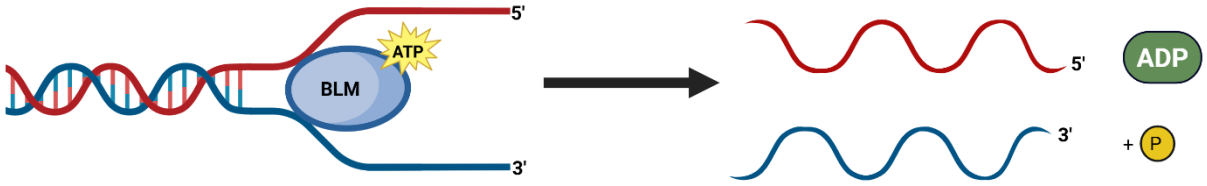

Figure 1: BLM mechanism of action.

BLM is a helicase that unwinds DNA substrates. This reaction involves ATP, which is converted into ADP. The levels of ADP can be quantified using ADP-Glo™ and the luminescence signal is directly proportional to the level of BLM ATPase activity.

Background

The BLM helicase, also known as Bloom syndrome protein, is a key enzyme involved in DNA replication and repair (DDR). The BLM helicase is a member of the RecQ family of helicases, which are evolutionarily conserved and found in many organisms, including bacteria, yeast, and humans. It catalyzes the unwinding of duplex DNA with 3' to 5' directionality, driven by the energy generated from ATP hydrolysis. BLM plays a crucial role in maintaining genomic stability by unwinding DNA structures during processes such as DNA replication, recombination, and repair. Mutations in the BLM gene can lead to Bloom syndrome, a rare genetic disorder characterized by growth deficiency, sun-sensitive skin lesions, and an increased risk of cancer. High expression of BLM is found in glioblastoma, and it was found that inhibition of its activity leads to increased susceptibility to treatment with drugs targeting other proteins involved in DDR, such as PARP1 (poly-ADP ribosylation protein 1). The use of BLM inhibitors as part of a combinatory therapeutic approach may open new avenues of treatment in cancer therapy.

Applications

- Screen or titrate small molecule inhibitors or antagonists that affect ATPase activity of BLM in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102130	BLM, GST-Tag*	10 µg	-80°C
82547	DR-04 Buffer	4 ml	-20°C
82954	DR Substrate 5 (non-fluorogenic)	5 µl	-80°C
83012	200 mM ATP	25 µl	-20°C
82735	0.5 M DTT	200 µl	-20°C
82545	White non-binding low volume 96-well plate	1	Room Temperature

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

Materials Required but Not Supplied

Name	Ordering Information
ADP-Glo™ Kinase Assay	Promega #V6930
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	

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.

Assay Principle

The **ADP-Glo™ Kinase Assay (Promega #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear to 1 mM ATP.

Contraindications

- The final concentration of DMSO in the reaction should not exceed 1%.

Assay Protocol

- All samples and controls should be performed in duplicates.
- 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 ML216 (MedChemExpress Cat. #HY-12342) 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).

1. Prepare **Complete DR-04 Buffer** by adding 10 µl of **0.5 M DTT** to 1 ml of **DR-04 Buffer** and mix well.
2. Dilute **Complete DR-04 Buffer** 4-fold with distilled water. Mix well. This makes **1x Assay Buffer**.
3. Thaw **BLM** on ice. Briefly spin the tube containing the protein to recover its full content.
4. Dilute **BLM** to 5.7 ng/µl with 1x Assay Buffer. You will need 17.5 µl per well.
5. Add 17.5 µl of diluted BLM to all wells.

6. Prepare the **Test Inhibitor** (2.5 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 25 µl.

   6.1 If the Test Inhibitor is water-soluble, prepare a serial dilution in 1x Assay Buffer at concentrations 10-fold higher than the final desired concentrations. The 1x Assay Buffer is the Diluent Solution.

   OR

   6.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at 100-fold the highest desired concentration. Then dilute it 10-fold in 1x Assay buffer to prepare the highest concentration of the 10-fold intermediate solution. The concentration of DMSO 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 in the assay should not exceed 1%.

7. Add 2.5 µl of **Test Inhibitor** to the "Test Inhibitor" wells.

8. Add 2.5 µl of **Diluent Solution** to the "Blank" and "Positive Control" wells.

9. Pre-incubate the plate for 60 minutes at Room Temperature (RT).

10. Thaw **DR Substrate 5** on ice. Briefly spin the tube containing the substrate to recover its full content of the tube.

11. Dilute 167-fold the **DR Substrate 5** with 1x Assay Buffer. You will need 2.5 µl/well for the "Test Inhibitor" and "Positive Control" wells.

12. Add 2.5 µl/well of **diluted DR Substrate 5** to the "Test Inhibitor" and "Positive Control" wells.

13. Add 2.5 µl of **1x Assay Buffer** to the "Blank" wells.

14. Thaw **200 mM ATP** and keep it on ice.

15. Dilute **200 mM ATP** 20-fold in 1x Assay Buffer, to a concentration of 10 mM. You will need 2.5 µl/well.

Note: Aliquot any unused ATP into single use aliquots (minimum volume of 5 µl/ aliquot) and store immediately at -80°C.

16. Initiate the reaction by adding 2.5 µl of diluted **ATP** (10 mM) to all wells.

Component	Blank	Positive Control	Test Inhibitor
Diluted BLM (5.7 ng/µl)	17.5 µl	17.5 µl	17.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
Preincubate 60 minutes at RT			
1x Assay Buffer	2.5 µl	-	-
Diluted DR Substrate 5	-	2.5 µl	2.5 µl
Diluted ATP (10 mM)	2.5 µl	2.5 µl	2.5 µl
Total	**25 µl**	**25 µl**	**25 µl**

17. Briefly shake the plate and incubate for 30 minutes at Room Temperature (RT).

18. Thaw the ADP-Glo™ reagent.

19. At the end of the 30-minute reaction, add 25 µl of ADP-Glo™ reagent to each well.

20. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.

21. Thaw the Kinase Detection Reagent.

22. Add 50 µl of Kinase Detection reagent to each well.

23. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.

24. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

25. The “Blank” value is subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, 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: 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

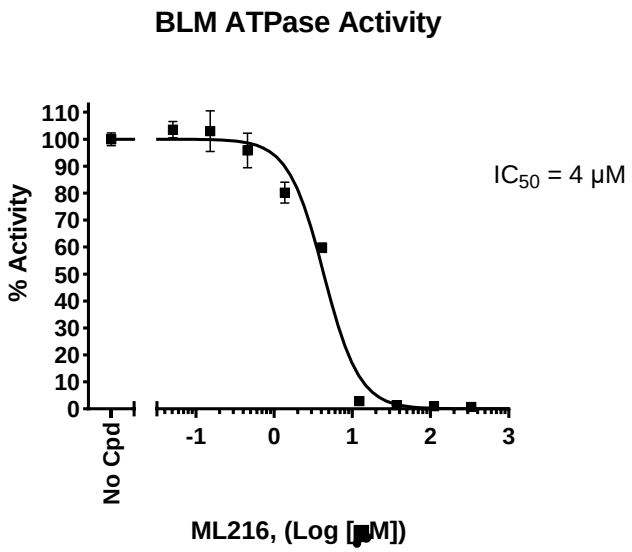

Figure 2: Inhibition of BLM ATPase activity by ML216.
Inhibition of BLM was evaluated in the presence of increasing concentrations of the BLM inhibitor ML216 (MedChemExpress Cat. #HY-12342). Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Chen, X., et al., 2021. eLife 10 :e65339.
Wojnicki K., et al., 2023 Cell Death Discovery 9 :157.

Related Products

Products	*Catalog #*	*Size*
BLM Helicase Activity Assay Kit	82512	96 reactions
WRN Helicase Activity Assay Kit	78852	96 reactions/ 384 reactions
WRN ATPase Activity Assay Kit	83017	96 reactions
WRN, GST-Tag Recombinant	102264	100 µg

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