Description

The BLM Helicase Activity Assay Kit is a fluorogenic assay designed for screening and profiling of BLM (Bloom syndrome protein) antagonists/inhibitors by monitoring their inhibitory effects on BLM-catalyzed DNA unwinding. The BLM Helicase Activity Assay Kit comes in a convenient 96-well format, with enough purified recombinant BLM (amino acids 630-1300), ATP, DNA substrate, assay buffer, and additives for 100 reactions.

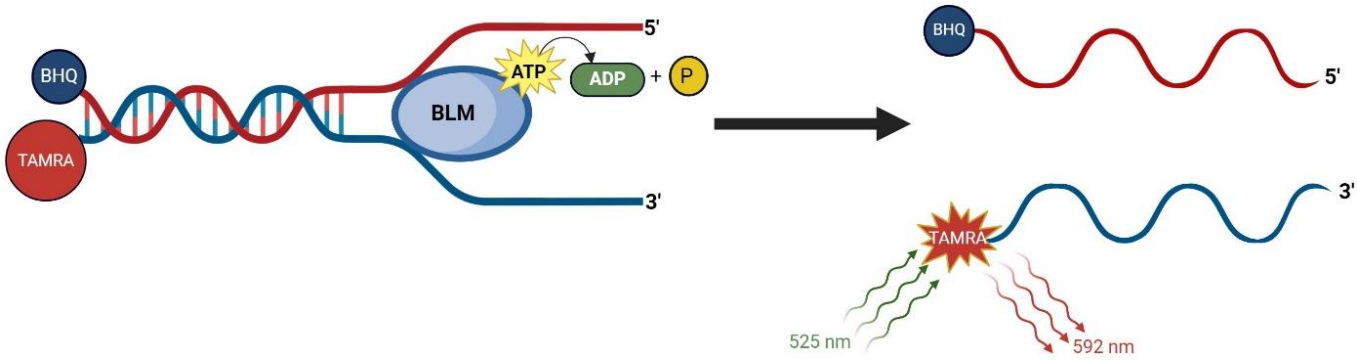

Figure 1: Illustration of the assay principle.

BLM is incubated with the fluorescent DNA substrate in the presence of ATP. The DNA substrate is conjugated on one strand with the TAMRA (tetramethylrhodamine) fluorophore, and on the other strand with BHQ (Black Hole Quencher) which effectively quenches TAMRA fluorescence due to their proximity within the DNA double strand. BLM unwinding of the DNA probe separates the two strands, releasing TAMRA fluorescence. BLM activity, therefore, results in a proportional increase in fluorescence.

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 helicase activity of BLM in high throughput screening (HTS) applications.
- Counter-screen for compounds that affect the exonuclease activity of BLM.

Supplied Materials

Catalog #	Name	Amount	Storage
102130	BLM, GST-Tag*	10 µg	-80°C
82547	DR-04 Buffer**	4 ml	-20°C
82645	DR Substrate 5***	20 µl	-80°C
83012	200 mM ATP	50 µl	-20°C
82735	0.5 M DTT	200 µl	-20°C
79685	Black, low binding 96-well plate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*
*** Previously named 4x BLM Buffer*
**** Previously named DNA Substrate*

Materials or Instruments Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Orbital shaker
- Fluorescent microplate reader capable of reading λex/em=525 nm/592 nm.

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed. Avoid multiple freeze/ thaw cycles!

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 final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Negative Control”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend using ML216 as an internal control for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- 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 each 1 ml of **RD-04 Buffer** and mix well.

2. Dilute 1 ml of **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 the full content of the tube.

4. Dilute **BLM** to 2.0 ng/µl with 1x Assay Buffer (40 µl/well).

5. Add 40 µl of diluted BLM to the "Positive Control" and "Test Inhibitor" wells.

6. Add 40 µl of 1x Assay Buffer to the "Negative Control" wells.

7. Prepare the **Test Inhibitor** (5 µl per 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.

   7.1 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.

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

   Or

   7.2 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%.

8. Add 5 µl of **Test Inhibitor** dilutions to the "Test Inhibitor" wells.

9. Add 5 µl of **Diluent Solution** to the "Negative Control" and "Positive Control" wells.

10. Briefly shake the plate and incubate for 60 minutes at Room Temperature (RT).

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

12. Dilute 200 mM ATP 5-fold with 1x Assay Buffer to a concentration of 40 mM (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.

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

14. Dilute 16.7-fold the DR Substrate 5 with 1x Assay Buffer (2.5 µl/well).

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

15. Prepare a **Master Mix** (5 µl/well): N wells × (2.5 µl of diluted DR Substrate 5 + 2.5 µl of diluted ATP).

16. Start the reaction by adding 5 µl of **Master Mix** to each well. Protect your samples from direct exposure to light, shake briefly and incubate at RT for 25 minutes or perform kinetic analysis.

    Note: For kinetic analysis use kinetic mode with a recommended kinetic interval of 5 minutes.

Component	Negative Control	Positive Control	Test Inhibitor
1x Assay Buffer	40 µl	-	-
Diluted BLM (2.0 ng/µl)	-	40 µl	40 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Pre- incubate 60 minutes RT			
Master Mix	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**

17. Read the plate in a fluorescent microplate reader capable of reading λex/λem=525 nm/592 nm.

18. Subtract the "Negative Control" value from all other values.

Example Results

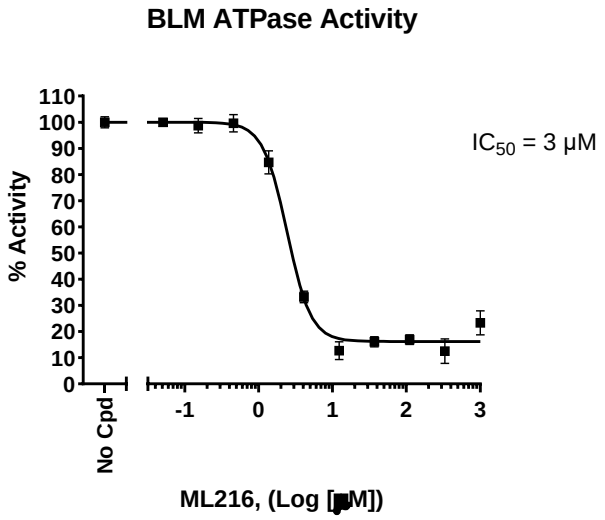

Figure 2: Inhibition of BLM helicase activity by ML216.
Inhibition of BLM was evaluated in the presence of increasing concentrations of the BLM inhibitor ML216 (MedChemExpress #HY-12342). BLM enzyme was preincubated with the inhibitor for 1h prior initiation of the reaction. Results are expressed as 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.

Reference

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

Related Products

Products	*Catalog #*	*Size*
WRN, GST-Tag Recombinant	101264	100 µg
WRN Helicase Activity Assay Kit	78852	96 reactions
Dicer, FLAG-Tag Recombinant	101532	20 µg/100 µg
Dicer Fluorogenic Assay Kit	78855	384 reactions

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