Description

The WRN Helicase Activity Assay is a fluorogenic assay designed for screening and profiling of WRN (Werner Syndrome ATP-dependent Helicase) antagonists/inhibitors by monitoring their effect on WRN-catalyzed DNA unwinding. WRN Helicase Activity Assay Kit comes in a convenient 96-well format, with contains enough purified recombinant WRN, ATP, WRN substrate, assay buffer and additives for 100 reactions.

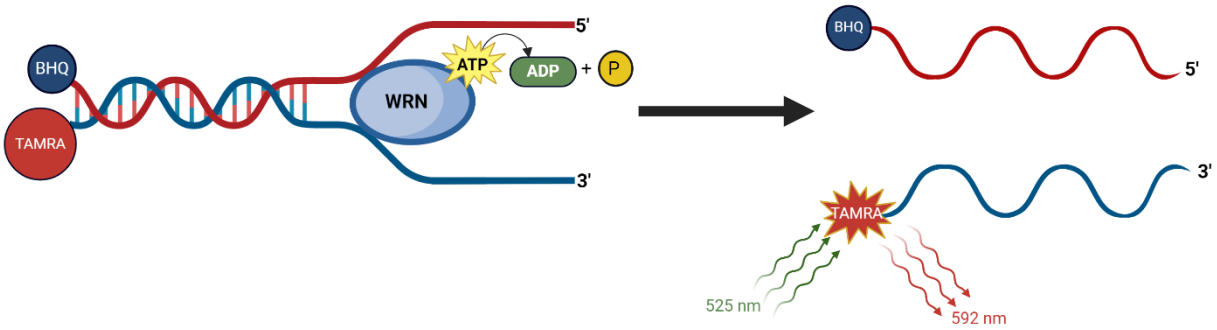

Figure 1: Illustration of the assay principle.

The DNA probe 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. WRN unwinding of the DNA probe separates the two strands, releasing TAMRA fluorescence. WRN activity, therefore, results in a proportional increase in fluorescence.

Background

The WRN gene was first discovered as the gene mutated in Werner syndrome, a recessive genetic disorder characterized by segmental progeria and elevated cancer predisposition. WRN is a multifunctional enzyme with helicase and exonuclease activities and plays roles in various cellular processes crucial for the maintenance of genome stability, including DNA replication, transcription, DNA repair, and telomere maintenance. WRN depletion causes cell cycle arrest, DNA damage, mitotic defects, chromosome shattering, and apoptosis. Loss of heterozygosity involving the *WRN* loci at chromosome 8p11.2-p12 occurs frequently in many different cancers, pointing to its role as a tumor suppressor gene. Small molecule inhibitors of WRN can be used to induce synthetic lethality and offer a new therapeutical approach for cancer treatment.

Applications

Screen small molecule inhibitors or antagonists that affect helicase activity of WRN in high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
101264	WRN (517-1093), GST-Tag*	2 x 10 µg	-80°C
82547	4x WRN Buffer	4 ml	-20°C
82645	WRN Substrate	20 µl	-80°C
	ATP (200 mM)	50 µl	-20°C
	0.5 M DTT	200 µl	-20°C
79685	Black, low binding plate	1	Room Temperature

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

Materials Required but Not Supplied

Fluorescent microplate reader capable of reading λexc/λem=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.

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 reaction 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 with the assay results.

Assay Protocol

- All samples and controls should be performed in duplicates.
- The assay should include "Negative Control", "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 HRO761 (#82691) 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.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Prepare **4x Complete WRN Buffer**: add 40 µl of 0.5 M DTT to 4 ml of **4x WRN Buffer** and mix well.

2. Prepare **1x Complete WRN Buffer**: dilute 1 ml of **4x Complete WRN Buffer** 4-fold with distilled water. Mix well.

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3. Thaw **WRN** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

4. Dilute **WRN** to 5 ng/µl with **1x Complete WRN Buffer**. You will need 40 µl per well.

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

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

7. 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.

   7.1 If the Test Inhibitor is water-soluble, prepare a serial dilution in 1x Complete WRN Buffer at concentrations 10-fold higher than the final desired concentrations. The 1x Complete WRN Buffer is the 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 Complete WRN 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 Complete WRN buffer, to keep the concentration of DMSO constant.

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

   Caution: The final concentration of DMSO in the assay should not exceed 1%.

8. Add 5 µl of Test Inhibitor 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 15-20 minutes at Room Temperature (RT).

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

12. Dilute **ATP** in 1x Complete WRN Buffer 5-fold to a concentration of 40 mM. You will need 2.5 µl/well.

    Note: Aliquot any unused ATP into single use aliquots and store immediately at -80°C.

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

14. Dilute **WRN Substrate** 12.5-fold in 1x Complete WRN Buffer. You will need 2.5 µl/well.

    Note: Aliquot any unused WRN substrate into single use aliquots and store immediately at -80°C.

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15. Prepare a **Master Mix** by combining diluted **ATP** (40 mM) and diluted **WRN substrate** at a 1:1 ratio as follows: N wells × (2.5 µl of diluted WRN substrate + 2.5 µl of diluted ATP).

16. Initiate the reaction by adding 5 µl of Master Mix, prepared as described above, to all the wells.

Component	Negative Control	Positive Control	Test Inhibitor
1x Complete WRN Buffer	40 µl	-	-
Diluted WRN (5 ng/µl)	-	40 µl	40 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
	15-20 minutes at RT		
Master Mix	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**

17. Briefly shake the plate and immediately place into a fluorescent microplate reader capable of reading λexc/λem=525 nm/592 nm.

18. Read end-point fluorescence after 25 minutes or use kinetic mode by reading several time points after the addition of the Master Mix. Recommended time interval is 5 minutes.

19. Calculate results by subtracting the "Negative Control" value from the other values.

Example Results

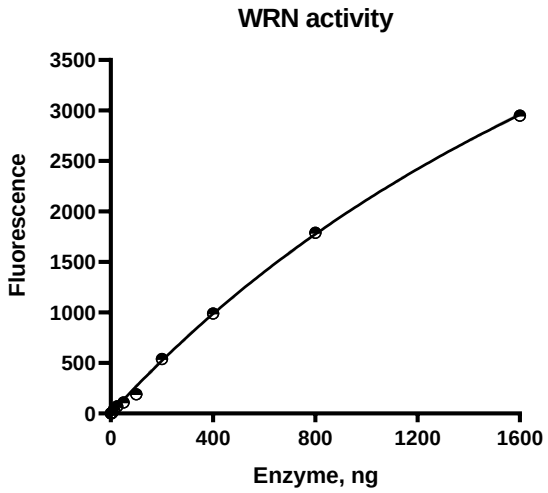

Figure 2: Titration of WRN.
Fluorescence was measured in the presence of increasing amounts of WRN and 2 mM ATP.

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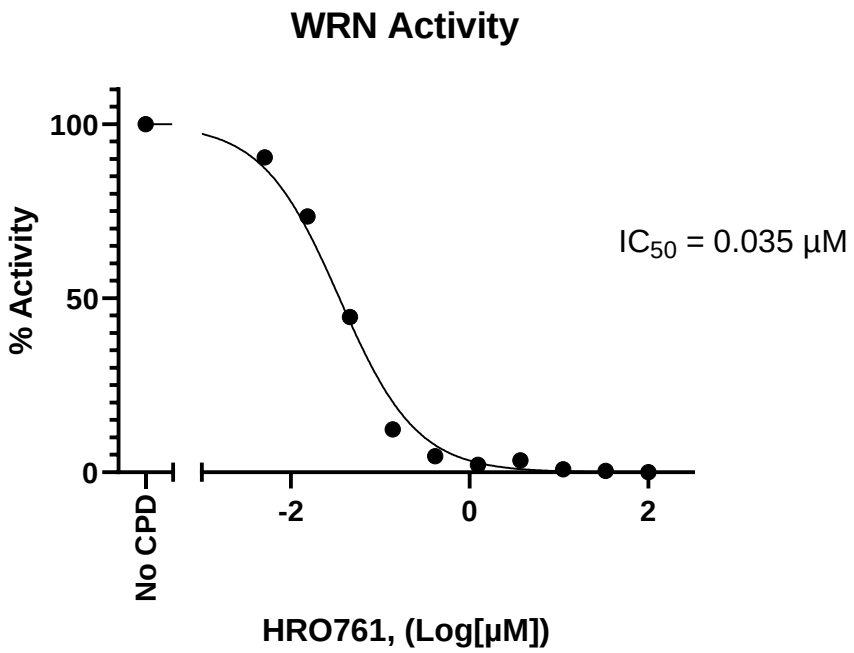

Figure 3: Effect of WRN inhibitor on helicase activity.
Inhibition of WRN was evaluated in the presence of increasing concentrations of the WRN inhibitor HRO761 (#82691). Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

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 further questions, please email support@bpsbioscience.com

References
Mendoza, O., *et al.* 2015. *Nucleic Acids Res* 43(11): e71.
Van Wietmarschen, N., *et al.* 2021. *Curr Opin Genet Dev* 71: 34-38.

Related Products

Products	*Catalog #*	*Size*
WRN, GST-Tag Recombinant	101264	100 µg
Dicer, FLAG-Tag Recombinant	101532	20 µg/100 µg
CHD2, GST-Tag Recombinant	55005	25 µg/100 µg

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