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 384-well format, with contains enough purified recombinant WRN (amino acids 517-1093), ATP, WRN substrate, assay buffer and additives for 400 reactions.

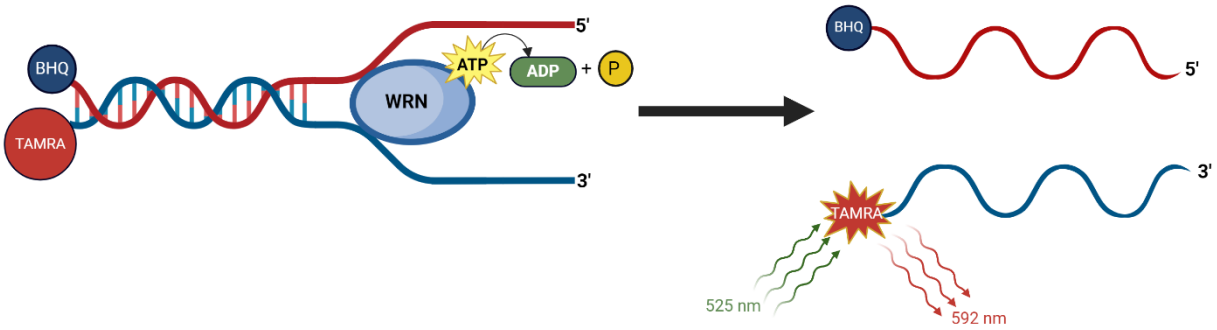

Figure 1: Illustration of the assay principle.

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.

Supplied Materials

Catalog #	Name	Amount	Storage
101264-KC10	WRN (517-1093), GST-Tag*	4 x 10 µg	-80°C
82547-KC4	DR-04 Buffer**	2 x 4 ml	-20°C
82645-KC20	DR Substrate 5	2 x 20 µl	-80°C
83012-KC50	200 mM ATP	2 x 50 µl	-20°C
82735-KC200	0.5 M DTT	2 x 200 µl	-20°C
79961	Low binding, black 384-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*
***Previously known as 4x WRN Buffer.*
**** Previously known as WRN Substrate.*

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Fluorescent microplate reader capable of reading λexc/λem=525 nm/592 nm
- Orbital shaker

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 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 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 **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 1 ml of **Complete DR-04 Buffer** 4-fold with distilled water. Mix well. This makes **1x Assay Buffer**.

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

4. Dilute **WRN** to 6.7 ng/µl with **1x Assay Buffer**. You will need 15 µl per well.

5. Add 15 µl of **diluted WRN** to the "Positive Control" and "Test Inhibitor" wells.

6. Add 15 µl of 1x Assay Buffer to the "Negative Control" wells.

7. Prepare the **Test Inhibitor** (5 µl/well): for a titration prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   7.1 If the Test Inhibitor is water-soluble, prepare a serial dilution in 1x Assay Buffer at concentrations 5-fold higher than the final desired concentrations.

   For the positive and negative controls, use 1x Assay Buffer (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 20-fold in 1x Assay buffer to prepare the highest concentration of the 5-fold intermediate solution. The concentration of DMSO is now 5%.

   Prepare serial dilutions of the Test Inhibitor at concentrations 5-fold higher than the desired final concentrations using 5% DMSO in 1x Assay buffer, to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 5% DMSO in 1x Assay 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).

    Note: Some inhibitors may require longer pre-incubation time.

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

12. Dilute **200 mM ATP** with 1x Assay Buffer 10-fold to a concentration of 20 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 substrate to recover the full content of

the tube.

14. Dilute **DR Substrate 5** 25-fold in 1x Assay Buffer (2.5 µl/well).

    Note: Aliquot any unused 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	15 µl	-	-
Diluted WRN (6.7 ng/µl)	-	15 µl	15 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	**25 µl**	**25 µl**	**25 µl**

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

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

Example Results

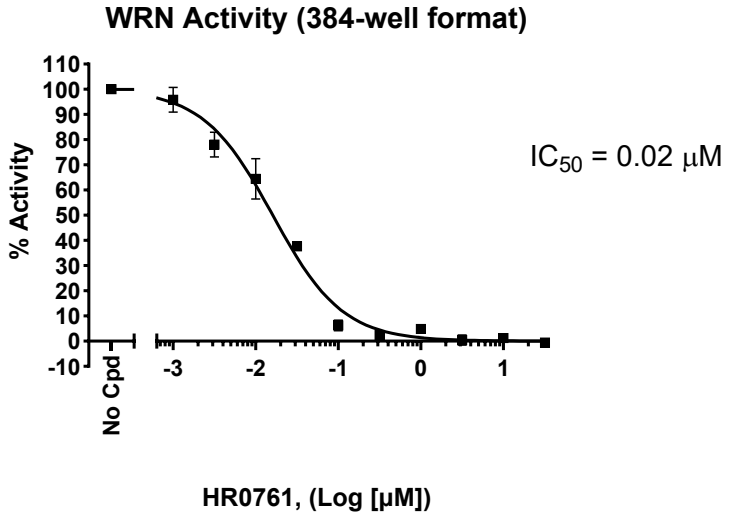

Figure 2: Effect of WRN inhibitor on helicase activity.
Inhibition of WRN was evaluated in the presence of increasing concentrations of the WRN inhibitor HRO761 (#82691). Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

Data shown is representative.

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

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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