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Produktinformation

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- Zellkultur & Verbrauchsmaterial
- 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 WRN ATPase Activity Assay Kit is designed for screening and profiling of WRN (Werner Syndrome ATP-dependent Helicase) 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 WRN. WRN ATPase Activity Assay Kit comes in a convenient 96-well format, with enough purified recombinant WRN (amino acids 517-1093), ATP, WRN substrate, assay buffer and additives for 100 reactions.

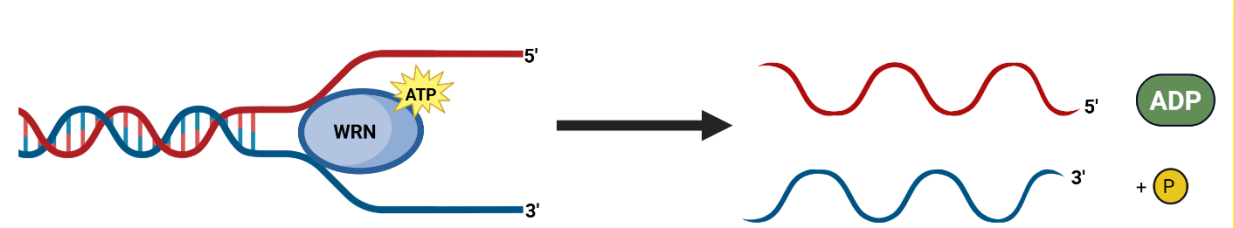

Figure 1: WRN mechanism of action.
WRN is a helicase that unwinds DNA substrates. This reaction involves ATP, which is converted into ADP. The levels of ADP can be quantified using ADP-Glo™ and the luminescence signal is directly proportional to the level of WRN ATPase activity.

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	WRN, 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 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 **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 5.7 ng/µl with 1x Assay Buffer. You will need 17.5 µl per well.
5. Add 17.5 µl of diluted WRN 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).

Caution: 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 20 minutes at Room Temperature (RT).

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

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

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 with 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 WRN (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	-
preincubation 20 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

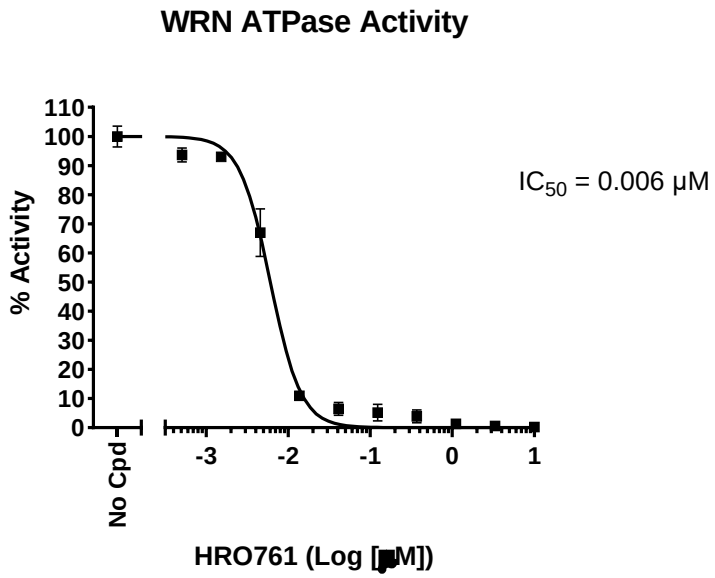

Figure 2: Inhibition of WRN ATPase activity by HRO761.
Inhibition of WRN was evaluated in the presence of increasing concentrations of the WRN inhibitor HRO761 (#82691). Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

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 Helicase Activity Assay Kit	78852	96 reactions/ 384 reactions
BLM Helicase Activity Assay Kit	82512	96 reactions
BLM ATPase Activity Assay Kit	83018	96 reactions
BLM, GST-Tag Recombinant	102130	10 µg/ 25 µg

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