Description

The DHX9 ATPase Activity Assay Kit is designed for the screening and profiling of DHX9 (DExH-box helicase 9) antagonists/inhibitors by monitoring their effect on the conversion of ATP to ADP using ADP-Glo™ as a detection reagent. ATP to ADP conversion occurs during DNA or RNA unwinding by the ATP dependent helicase domain of DHX9. DHX9 ATPase Activity Assay Kit comes in a convenient 96-well format, with contains enough purified recombinant DHX9 (amino acids 150-1150), ATP, DHX9 substrate, assay buffer and additives for 100 reactions.

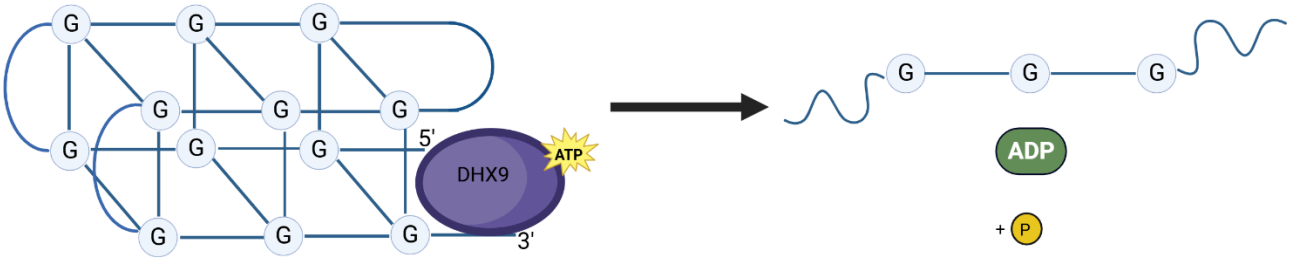

Figure 1: DHX9 mechanism of action.

DHX9 is a helicase that unwinds quadruplex DNA and RNA 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 DHX9 helicase activity.

Background

DHX9 (DExH-Box Helicase 9), also known as RNA Helicase A or Nuclear Helicase II (NDHII), is a member of the DEAH-containing family of RNA helicases. The encoded protein is an enzyme that catalyzes the ATP-dependent unwinding of double-stranded RNA and DNA-RNA complexes. This protein localizes mainly to the nucleus but can migrate to the cytoplasm, and functions as a transcriptional regulator. It is ubiquitously expressed and abundant. It interacts with many proteins, such as PRMT1 (protein arginine N-methyltransferase 1) and WRN (Werner Syndrome ATP-dependent Helicase). This protein may also be involved in the expression and nuclear export of retroviral RNAs, and is studied for its roles in cancer progression, antiviral immune response, and aging. DHX9 has become a relevant therapeutic target for diseases such as MM (multiple myeloma), showing promising results.

Applications

Screen small molecule inhibitors or antagonists that affect ATPase activity of DHX9 in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102248	DHX9, FLAG-Tag*	4 µg	-80°C
78856	U2 Assay Buffer	10 ml	-20°C
82972	DHX9 Substrate (non-fluorogenic)	25 µl	-80°C
82509	4 mM ATP	250 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 the 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%.
- Some compounds may interfere with the results, depending on their color, 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 “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 GTPαS (Jena Bioscience #NU-4095) 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. Thaw **DHX9** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

2. Dilute **DHX9** to 2.2 ng/µl with **U2 Assay Buffer** (17.5 µl/well).

3. Add 17.5 µl of **diluted DHX9** to all wells.

4. 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.

   4.1 If the Test Inhibitor is water-soluble, prepare a serial dilution in U2 Assay Buffer at concentrations 10-fold higher than the final desired concentrations.

   For positive and negative controls use U2 Assay Buffer (Diluent Solution).

   OR

   4.2 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 U2 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 U2 Assay Buffer, to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in U2 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%.

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

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

7. Pre-incubate the plate for 20-60 minutes at Room Temperature (RT).

8. Thaw **DHX9 Substrate** on ice. Briefly spin the tube containing the DHX9 substrate to recover the full DNA content of the tube.

9. Dilute **DHX9 Substrate** 25-fold with U2 Assay Buffer. You will need 2.5 µl/well for the "Test Inhibitor" and "Positive Control" wells.

10. Add 2.5 µl of **diluted DHX9 Substrate** to the "Test Inhibitor" and "Positive Control" wells.

11. Add 2.5 µl **U2 Assay Buffer** to the "Blank" wells.

12. Thaw **4 mM ATP** and keep it on ice.

13. Dilute 4 mM ATP 4-fold with U2 Assay Buffer (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.

14. Initiate the reaction by adding 2.5 µl of **diluted ATP** (1 mM) to all wells.

Component	Blank	Positive Control	Test Inhibitor
Diluted DHX9 (2.2 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 20-60 minutes at RT			
U2 Assay Buffer	2.5 µl	-	-
Diluted DHX9 Substrate	-	2.5 µl	2.5 µl
Diluted ATP (1 mM)	2.5 µl	2.5 µl	2.5 µl
Total	**25 µl**	**25 µl**	**25 µl**

15. Incubate for 2 hours at RT.

16. Thaw the ADP-Glo™ reagent.

17. At the end of the 2-hour reaction, add 25 µl of ADP-Glo™ reagent to each well.

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

19. Thaw the Kinase Detection Reagent.

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

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

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

23. The "Blank" value should be 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

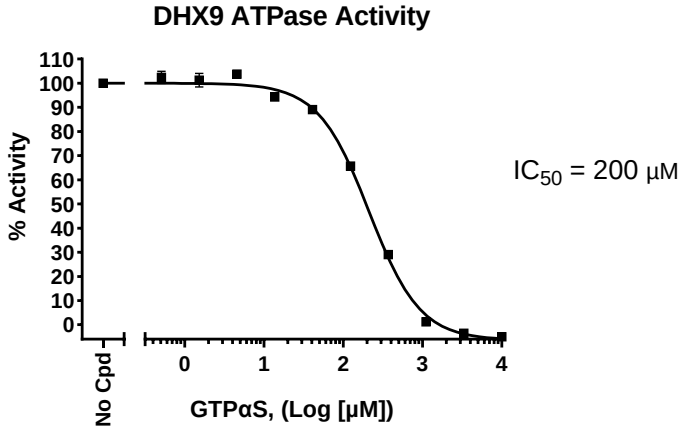

Figure 2: Inhibition of DHX9 ATPase activity by GTPαS.
Inhibition of DHX9 was evaluated in the presence of increasing concentrations of the inhibitor GTPαS (Jena Bioscience #NU-4095). Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

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
Castro J., *et al.*, 2025 *Cancer Res* 85(4):758-776.

Related Products

Products	*Catalog #*	*Size*
WRN, GST-Tag Recombinant	101264	100 µg
WRN Helicase Activity Assay Kit	78852	96 reactions/ 384 reactions
Dicer, FLAG-Tag Recombinant	101532	20 µg/100 µg
Dicer Fluorogenic Assay Kit	78855	384 reactions
CHD2, GST-Tag Recombinant	55005	25 µg/100 µg

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