Description

The DHX9 Helicase Activity Assay Kit is a fluorogenic assay designed for screening and profiling DHX9 (DExH-Box Helicase 9) antagonists/inhibitors by monitoring their effect on DHX9-catalyzed RNA unwinding. DHX9 Helicase 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.

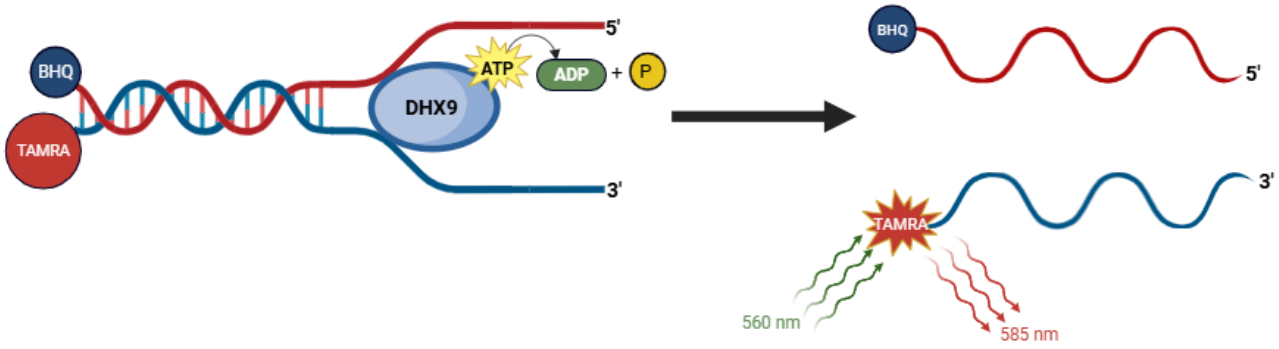

Figure 1: Illustration of the assay principle.

The RNA 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 RNA double strand. DHX9 unwinding of the RNA probe separates the two strands, releasing TAMRA fluorescence. DHX9 activity, therefore, results in a proportional increase in fluorescence.

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 helicase activity of DHX9 in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102248-KC4	DHX9, FLAG-Tag*	4 µg	-80°C
78856	U2 Assay Buffer	10 ml	-80°C
83685-KC15^{+}	DHX9 Substrate (Fluorogenic)	15 µl	-80°C
82509-KC250	4 mM ATP	250 µl	-80°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

- Adjustable micropipettor and sterile tips
- Fluorescent microplate reader capable of reading λexc/λem=560 nm/585 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 ATX968 (BPS Bioscience #83722) and DHX9-IN-3 (BPS Bioscience #83723) as internal controls. 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. Dilute **DHX9** to 1.1 ng/µl with **U2 Assay Buffer**. You will need 35 µl per well.

2. Add 35 µl of **diluted DHX9** to the "Positive Control" and "Test Inhibitor" wells.

3. Add 35 µl of **U2 Assay Buffer** to the "Negative Control" wells.

4. 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.

   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 the 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).

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

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

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

7. Briefly shake the plate and incubate for 30 minutes at Room Temperature (RT).

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

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

9. Dilute **4 mM ATP** with U2 Assay Buffer 16-fold to a concentration of 250 µM (5 µl/well).

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

10. Thaw **DHX9 Substrate (Fluorogenic)** on ice. Briefly spin the tube containing the substrate to recover its full content.

11. Dilute **DHX9 Substrate (Fluorogenic)** 40-fold with U2 Assay Buffer (5 µl/well).

    Note: Aliquot any unused substrate into single use aliquots (minimum volume of 5 µl/ aliquot) and store

immediately at -80°C.

12. Prepare a **Master Mix** (10 µl/well): N wells × (5 µl of diluted DHX9 Substrate (Fluorogenic) + 5 µl of diluted ATP).

13. Start the reaction by adding 10 µl of **Master Mix** to each well. Protect your samples from direct exposure to light, shake briefly and incubate at RT for 10-15 minutes or perform kinetic analysis.

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

Component	Negative Control	Positive Control	Test Inhibitor
U2 Assay Buffer	35 µl	-	-
Diluted DHX9 (1.1 ng/µl)	-	35 µl	35 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
	30 minutes at RT		
Master Mix	10 µl	10 µl	10 µl
Total	**50 µl**	**50 µl**	**50 µl**

14. Read the plate in a fluorescent microplate reader capable of reading λexc/λem =560 nm/585 nm with a bandwidth of 10 nm for both wavelengths.

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

Example Results

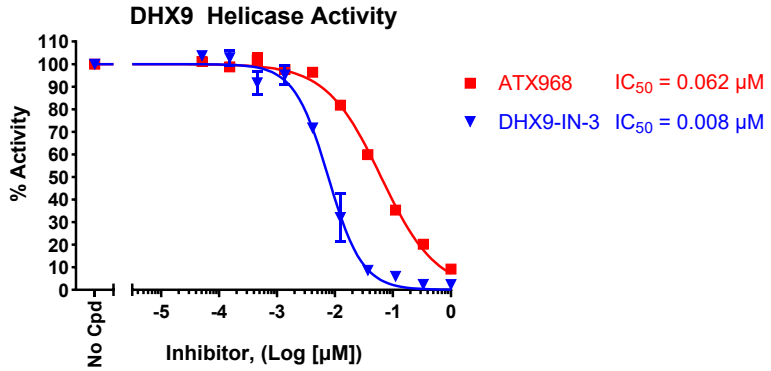

Figure 2: DHX9 helicase activity is inhibited by ATX968 and DHX9-IN-3.
Inhibition of DHX9 was evaluated in the presence of increasing concentrations of the DHX9 inhibitors ATX968 (#83722) and DHX9-IN-3 (#83723). Results are expressed as percent of control activity (measured in the absence of inhibitor 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

Version 101625