Description

The DNA Polymerase θ Assay Kit is a fluorogenic assay designed to measure DNA Polymerase θ activity for screening and profiling applications, utilizing the fluorescent nucleic acid dye GroovyGreen™ for the quantitation of double-stranded DNA (dsDNA) products. The kit comes in a convenient 96-well format and contains enough recombinant DNA Polymerase θ enzyme (amino acids 1792-2590), DR Substrate 3, dNTP mix and buffer for 100 enzymatic reactions.

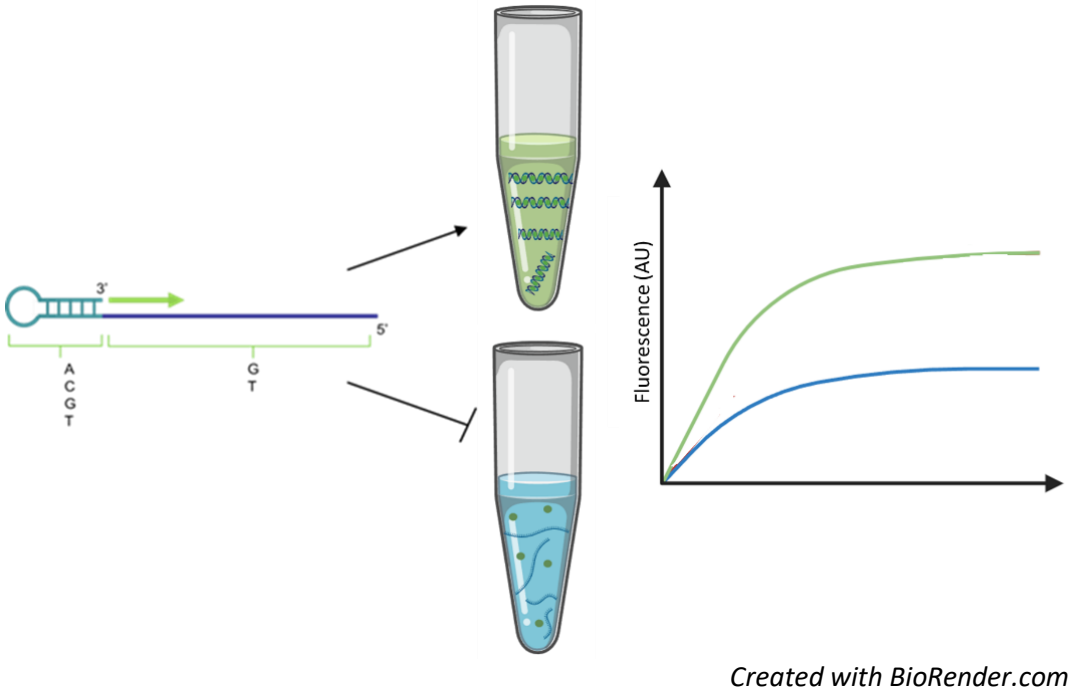

Figure 1: DNA Polymerase θ Assay Kit mechanism.

DNA Polymerase θ uses available nucleotides to synthesize a DNA strand complementary to the template strand, leading to the formation of double-stranded DNA molecules (dsDNA). The amount of dsDNA formed directly correlates to the polymerase activity and can be quantified by the addition of a fluorescent dsDNA-binding dye which distinguishes dsDNA from ssDNA and free nucleotides. Since the dye emits fluorescence only when bound to the target dsDNA, the fluorometric readouts are low in the presence of a polymerase inhibitor.

Background

Polymerases are the enzymes responsible for synthesizing nucleic acids. DNA Polymerase θ, also known as DNA polymerase subunit theta, POLQ, belongs to the Family A of DNA polymerases. In addition to its DNA polymerase activity, it acts on the theta-mediated end joining (TMEJ), which classifies it as a specialized polymerase. TMEJ is a DNA repair mode that is prone to errors, and in addition to DNA polymerase θ it involves PARP1 (poly polymerase 1) and DNA ligase III. Inhibition of DNA polymerase θ combined with a homologous recombination (HR) deficiency, as in BRCA (breast cancer type 1/2 susceptibility protein) deficient cells, is synthetic lethal. This makes its inhibitors candidates for HR deficient cancers. It can also enhance the effect of PARP inhibitors and be used in cases where resistance to PARP inhibitors has occurred. Further development of DNA polymerase θ inhibitors will bring new therapy options to cancer patients, potentially bypassing the dose-limiting toxicities seen when using PARP inhibitors combined with conventional chemotherapy.

Applications

- Screen molecules that inhibit polymerization activity of polymerase θ in drug discovery and high throughput screening (HTS) applications.
- Determine compound IC_{50}.

Supplied Materials

Catalog #	Name	Amount	Storage
101945	DNA Polymerase θ, His-Tag, SUMO-Tag Recombinant*	>1 µg	-80°C
	DR Substrate 3	1 µg	-80°C
	dNTP Substrate Mix	20 µl	-80°C
	10x DR Buffer 3A	2 x 1 ml	-20°C
	200x GroovyGreen™ Dye	25 µl	-20°C
	96-well black microplate	1	Room Temp

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

Materials Required but Not Supplied

Fluorescence plate reader capable of measurement at λex502/λem523 nm.

Stability

This assay kit contains DNA and 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 Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Negative Control", "Positive Control" and "Test Inhibitor" conditions.
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1. Prepare 1x Assay Buffer by diluting 10-fold **10x DR-3A Buffer** with distilled water.

2. Add 15 µl of 1x Assay Buffer to all wells, except the "Negative Control" wells.

3. Add 25 µl of 1x Assay Buffer to the "Negative Control" wells.

4. Dilute 28-fold the **DR Substrate 3** with 1x Assay Buffer.

5. Add 10 µl of diluted DR Substrate 3 to all wells.

6. Thaw **DNA Polymerase θ** on ice. Briefly spin the tube to recover the full content.

7. Dilute **DNA Polymerase θ** to 0.5 ng/µl (10 µl/well) with 1x Assay Buffer.

   Note: Keep the diluted protein on ice until use. Discard any unused diluted protein after use.

8. Add 10 µl of diluted DNA Polymerase θ to all wells except "Negative Control" wells.

9. 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.

   9.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   9.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using 1x Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   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).

   Note: The final concentration of DMSO should not exceed 1%.

10. Add 5 µl of inhibitor dilutions to each well designated "Test Inhibitor".

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

12. Preincubate the plate at Room Temperature (RT) for 30 minutes.

13. Dilute **dNTPs Substrate Mix** 50-fold in 1x Assay Buffer (10 µl /well).

14. Add 10 µl of diluted dNTPs Substrate Mix to all wells.

15. Incubate the plate at 37°C for 1 hour.

16. Dilute **200x GroovyGreen™ Dye** 200-fold with 1x Assay Buffer.

17. Add 50 µl of diluted dye to each well.

18. Read fluorescence intensity of the samples (lexcitation = 502/10 nm; lemission = 530/10 nm) in an appropriate microplate reader.

Component	Negative control	Positive Control	Test Inhibitor
Diluted Polymerase θ (0.5 ng/µl)	-	10 µl	10 µl
Diluted DR Substrate 3 (1:28)	10 µl	10 µl	10 µl
1x Assay Buffer	25 µl	15 µl	15 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted dNTPs Substrate Mix (1:50)	10 µl	10 µl	10 µl
Total	**50 µl**	**50 µl**	**50 µl**

Example Results

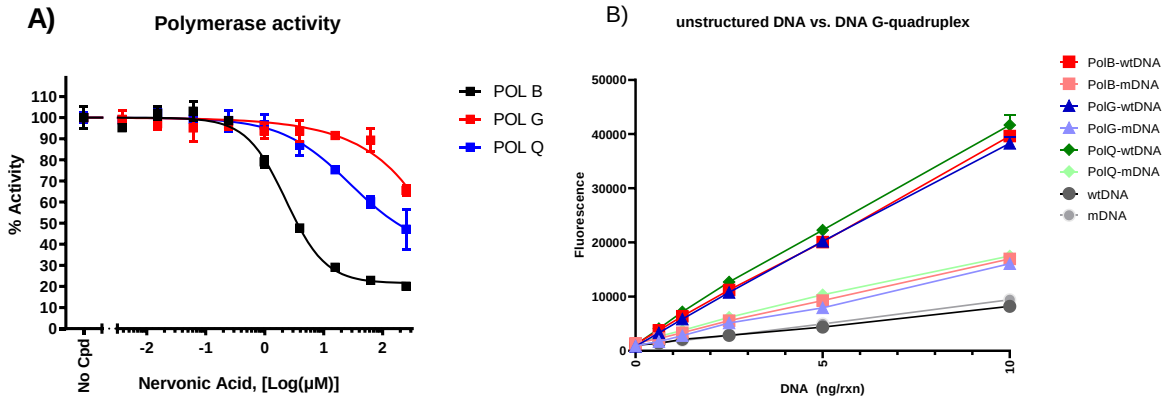

Figure 2. Inhibition of Pol β, γ and θ polymerase activity by nervonic acid and activity in the presence of G-quadruplex DNA variant.
The polymerization activity of several polymerases was measured in the presence of increasing concentrations of nervonic acid (Sigma-Aldrich #N1514) (A). Activity was also measured in the presence of DR Substrate 3 or its G-quadruplex variant (B).

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 all further questions, please email support@bpsbioscience.com

References
Berdis A., 2017, *Front. Mol. Biosci.* 4: https://doi.org/10.3389/fmolb.2017.00078
Zatreanu D., *et al.*, 2021 *Nature Communications* 12: 3636.

Related Products

Products	*Catalog #*	*Size*
DNA Polymerase β (POLB), His-Tag Recombinant	21000	100 µg/1 mg
DNA Polymerase β Assay Kit	82099	96 reactions
DNA Polymerase γ Assay Kit	82098	96 reactions