

**ProFoldin**

10 Technology Drive, Suite 40, Number 188  
Hudson, MA 01749-2791 USA  
Tel: (508)-735-2539 FAX: (508) 845-9258  
www.profoldin.com info@profoldin.com

## INSTRUCTIONS

### ProFoldin

## Human DNA Polymerase Theta (Pol $\theta$ ) ATPase Assay Kits

**Human DNA Polymerase Theta ATPase Assay Kit**

**Catalog No: HDPQ100K**

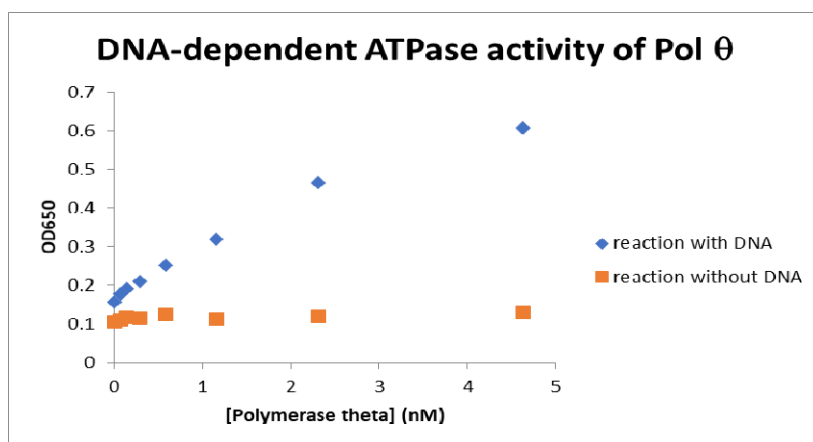
**Human DNA Polymerase Theta ATPase Assay Kit Plus**

**Catalog No: HDPQ100KE**

### Introduction

Human DNA polymerase theta (Pol  $\theta$ ) encoded by the POLQ gene plays a key role in the DNA repair process. Pol  $\theta$  is overexpressed in various cancers and is a validated anti-cancer drug target. It is a multiple-functional protein including an N-terminal ATPase-helicase domain and a C-terminal DNA polymerase domain. The human DNA polymerase theta ATPase assay is based on the DNA-dependent ATPase activity of the N-terminal ATPase-helicase domain. It is a colorimetric assay by detection of phosphate at the wavelength at 650 nm. The assay is performed in a 384-well or 96-well plate format. The assay can be used for detection of the DNA-dependent ATPase activity and inhibitors of human DNA polymerase theta.

The helicase activity assay of human DNA polymerase theta is also available. Please visit our website at <https://www.profoldin.com/dna-helicase.html>.



The **Human DNA Polymerase Theta ATPase Assay Kit (Catalog No. HDPQ100K)** includes all the assay kit components except the enzyme for 100 assays in a 384-well plate assay format: 400  $\mu$ l of 10 x Buffer, 33  $\mu$ l of 100 x DNA (20  $\mu$ M), 33  $\mu$ l of 100 x ATP (25 mM) and 5 ml of MPA3000 dye. The kit does not include the enzyme.

The **Human DNA Polymerase Theta ATPase Assay Kit Plus (Catalog No. HDPQ100KE)** includes all the assay kit components for 100 assays in a 384-well plate assay format: 400  $\mu$ l of 10 x Buffer, 33  $\mu$ l of 100 x DNA (20  $\mu$ M), 33  $\mu$ l of 100 x ATP (25 mM), 33  $\mu$ l of 100 x human DNA polymerase theta N-terminal domain and 5 ml of MPA3000 dye.

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# INSTRUCTIONS

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## Assay protocol

### 1. Time-course experiment

The optimal reaction time is the time that gives the maximum signal in the linear range. It is the reaction time used for the enzyme inhibition and it is determined by the time-course experiment.

#### (1) Reagent preparation

For each 10 assay reactions, prepare 297  $\mu$ l of premix composed of 257.4  $\mu$ l of H<sub>2</sub>O, 33  $\mu$ l of 10 x Buffer, 3.3  $\mu$ l of 100 x DNA and 3.3  $\mu$ l of 100 x pol Q. Also prepare 33  $\mu$ l of 10 x ATP by mixing 3.3  $\mu$ l of 100 x ATP and 29.7  $\mu$ l of water.

#### (2) Assay reaction and detection:

Mix 27  $\mu$ l of the premix with 3  $\mu$ l of the 10 x ATP in each well. Incubate the reaction mixture at 37°C. At various time points, add 45  $\mu$ l of the Dye MPA3000 into the 30  $\mu$ l of the reaction mixture and read light absorbance at 650 nm (OD<sub>650</sub>).

### 2. Enzyme inhibition

For each 10 assay reactions,

- (1) Add 0.6  $\mu$ l of 50 x inhibitor into each well.
- (2) Prepare 290.4  $\mu$ l of premix composed of 250.8  $\mu$ l of H<sub>2</sub>O, 33  $\mu$ l of 10 x Buffer, 3.3  $\mu$ l of 100 x DNA and 3.3  $\mu$ l of 100 x pol Q.
- (3) Prepare 33  $\mu$ l of 10 x ATP by mixing 3.3  $\mu$ l of 100 x ATP and 29.7  $\mu$ l of water.
- (4) Mix 26.4  $\mu$ l of the premix with the 0.6  $\mu$ l of inhibitor for 5 min.
- (5) Add 3  $\mu$ l of 10 x ATP to initiate the reaction.
- (6) Incubate the reaction at 37°C for the optimal time.
- (7) Add 45  $\mu$ l of the Dye MPA3000 into the 30  $\mu$ l of the reaction mixture and read the OD<sub>650</sub> values.

ProFoldin offers HTS assay development service. For more information, please visit our website at <http://www.profoldin.com/services.html>.

## Related Products

DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
HDP100KE1	Human DNA Polymerase Alpha Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
S2RPA100KE	SARS-CoV-2 RNA polymerase assay kit plus-100

For more information of drug targets and enzyme assays, please visit [www.profoldin.com](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).