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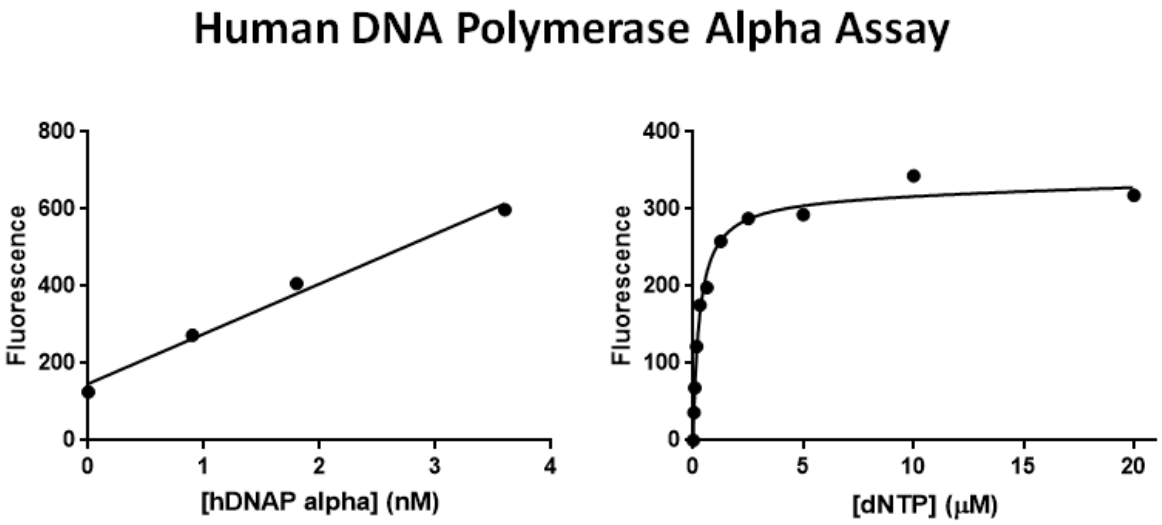
INSTRUCTIONS

ProFoldin
Human DNA Polymerase Alpha Assay Kits

Human DNA Polymerase Alpha Assay Kit **Catalog No: HDPA100K**
Human DNA Polymerase Alpha Assay Kit Plus **Catalog No: HDPA100KE1**

Introduction

Human DNA Polymerase Alpha (Pol α) is responsible for the initiation of DNA replication at origins of replication The Pol α enzyme consists of four subunits: the catalytic subunit POLA1, the regulatory subunit POLA2, and the small and the large primase subunits PRIM1 and PRIM2. The human DNA polymerase alpha assay is based on measurement of the DNA molecules synthesized by the DNA polymerase catalytic subunit. The assay is performed in a 384-well or 96-well plate format. The assay can be used for detection of DNA polymerase alpha activity and high throughput screen of human DNA polymerase alpha inhibitors.



The **Human DNA Polymerase Alpha Assay Kit (Catalog No. HDPA100K)** includes all the assay kit components except the enzyme for 100 assays in a 384-well plate assay format: 400 µl of 10 x Buffer, 33 µl of 100 x DNA template, 33 µl of 100 x dNTP mix, 1550 µl of 2 x Dye, 1550 µl of 50 mM EDTA. The kit does not include the enzyme.

The **Human DNA Polymerase Alpha Assay Kit Plus (Catalog No. HDPA100KE1)** includes all the assay kit components for 100 assays in 384-well plate assay format: 400 µl of 10 x Buffer, 33 µl of 100 x DNA template, 33 µl of 100 x dNTP mix, 30 µl of 100 x human DNA polymerase alpha, 1550 µl of 2 x Dye, 1550 µl of 50 mM EDTA.

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Assay protocol

The following assay protocol is based on the 384-well plate assay format (plate type: Matrix 4318 or alike). The reaction volume is 30 µl and the final assay volume is 60 µl. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60 µl and the final assay volume is 120 µl.

1. Reagent preparation:

(1) 10 x DNA: Dilute the 100 x DNA 10-fold with water. Each assay uses 3 µl of 10 x DNA.
(2) 10 x enzyme: The recommended final enzyme concentration is 4 to 8 nM in the assay.
(3) 10 x dNTP: Dilute the 100 x dNTP (10 mM) 10-fold with water. Each assay uses 3 µl of 10 x dNTP.
(4) 1 x dye: Dilute the 2 x fluorescence dye 2-fold with 50 mM EDTA. Each assay uses 30 µl of 1 x dye.

2. **Reaction**:

The total volume of each reaction mixture is 30 µl including 18 µl of H_2O, 3 µl of 10 x Buffer, 3 µl of 10 x DNA template, 3 µl of 10 x enzyme, 3 µl of 10 x dNTP. Incubate the reaction mixture at 37°C for 60 min. Note: The enzyme reaction rate will be slower if the assay is at a lower temperature.

3. **Detection**:

Mix 30 µl of the 1 x fluorescence dye with 30 µl of the reaction mixture for 5 min. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Assay Protocol for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at http://www.profoldin.com/services.html.

Related Products

DPA100KE	*E. coli* DNA Polymerase III Alpha Assay Kit Plus
DPA100KH	*H. influenzae* DNA polymerase Assay Kit Plus
DPA100KN	*S. pneumoniae* DNA polymerase Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100K	Human DNA Polymerase Gamma Assay Kit
RPA100KE	*E. coli* RNA Polymerase Assay Kit Plus
PNP100KE	*E. coli* Polynucleotide Phosphorylase (PNPase) Assay Kit Plus
T7RPA100K	T7 RNA Polymerase Assay Kit
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
RPA100KE	*E. coli* RNA Polymerase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus
PAR100KE	Human Poly (ADP-ribose) Polymerase-1 Assay Kit Plus

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.