



## ProFoldin

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# INSTRUCTIONS

## ProFoldin Human DNA Polymerase Alpha

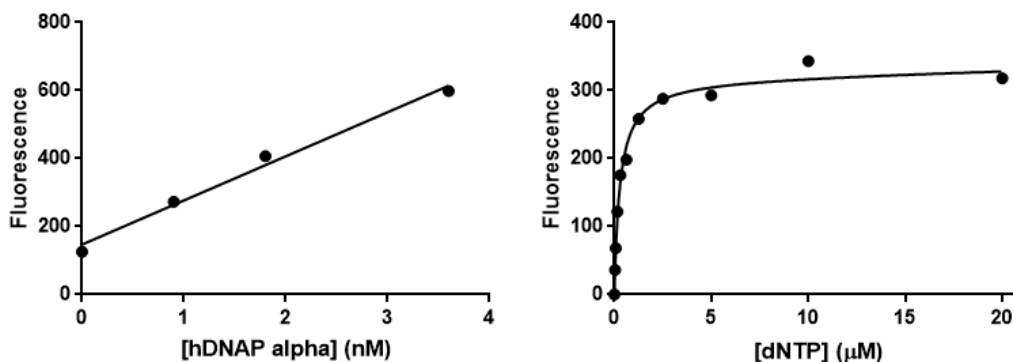
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**Human DNA Polymerase Alpha – for 100 assays**  
**Human DNA Polymerase Alpha – for 1000 assays**

**Catalog No: HDP A1-100**  
**Catalog No: HDP A1-1000**

Protein construct:	Wild-type human DNA polymerase alpha catalytic subunit purified from a baculovirus insect protein expression system.
MW:	170 kDa
Enzyme concentration:	4 $\mu$ M
DNA polymerase assay:	The DNA polymerase beta activity is measured by using the Human DNA Polymerase Assay Alpha kit (Catalog No. HDP A100K).
Storage temperature:	-20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution:	Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution.

### Human DNA Polymerase Alpha Assay



**Human DNA Polymerase Alpha – for 100 assays (Catalog No: HDP A1-100 )** includes 7  $\mu$ l of 4  $\mu$ M human DNA polymerase alpha catalytic subunit. It is sufficient for 100 assays.

**Human DNA Polymerase Alpha – for 1000 assays (Catalog No: HDP A1-1000)** includes 50  $\mu$ l of 4  $\mu$ M human DNA polymerase alpha catalytic subunit. It is sufficient for 1000 assays.

### Assay Protocol using Human DNA Polymerase Alpha Assay kit (Catalog No. HDP A100K)

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

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## 1. Reagent preparation:

- (1) 10 x DNA: Dilute the 100 x DNA 10-fold with water. Each assay uses 3  $\mu$ l 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  $\mu$ l of 10 x dNTP.
- (4) 1 x dye: Dilute the 2 x fluorescence dye 2-fold with 50 mM EDTA. Each assay uses 30  $\mu$ l of 1 x dye.

## 2. Reaction:

The total volume of each reaction mixture is 30  $\mu$ l including 18  $\mu$ l of H<sub>2</sub>O, 3  $\mu$ l of 10 x Buffer, 3  $\mu$ l of 10 x DNA template, 3  $\mu$ l of 10 x enzyme, 3  $\mu$ l 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  $\mu$ l of the 1 x fluorescence dye with 30  $\mu$ l 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	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
DPA100KH	<i>H. influenzae</i> DNA polymerase Assay Kit Plus
DPA100KN	<i>S. pneumoniae</i> DNA polymerase Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100K	Human DNA Polymerase Gamma Assay Kit
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
PNP100KE	<i>E. coli</i> Polynucleotide Phosphorylase (PNPase) Assay Kit Plus
T7RPA100K	T7 RNA Polymerase Assay Kit
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
RPA100KE	<i>E. coli</i> 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](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).

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