



ProFoldin

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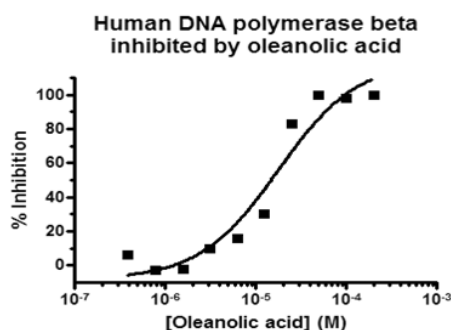
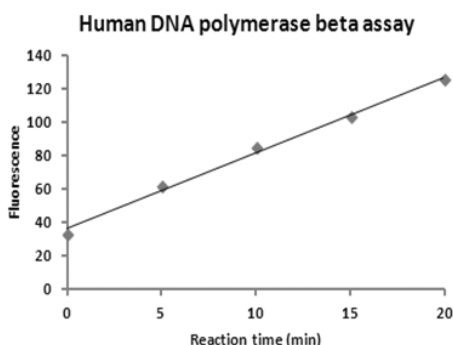
INSTRUCTIONS

ProFoldin Human DNA Polymerase Beta

Human DNA Polymerase Beta – for 100 assays
Human DNA Polymerase Beta – for 1000 assays

Catalog No: HDPB-100
Catalog No: HDPB-1000

Protein construct:	Wild-type human DNA polymerase beta purified from a baculovirus insect protein expression system.
MW:	39 kDa
Enzyme concentration:	10 μ M, 10,000 U/ml
DNA polymerase assay:	The DNA polymerase beta activity is measured by using the Human DNA Polymerase Assay kit (Catalog No. DPB100K).
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 Beta – for 100 assays (Catalog No: HDPB-100) includes 7 μ l of 10 μ M human DNA polymerase beta (1000 x stock, 10,000 U/ml). It is sufficient for 100 assays.

Human DNA Polymerase Beta – for 1000 assays (Catalog No: HDPB-1000) includes 50 μ l of 10 μ M human DNA polymerase beta (1000 x stock, 10,000 U/ml). It is sufficient for 1000 assays.

Assay Protocol using Human DNA Polymerase Assay kit (Catalog No. DPB100K)

1. Reagent preparation:

DNA, dTNP mix and dye are diluted with water. The DNA polymerase is diluted with 1 x assay buffer.

2. Reaction:

The total volume of each reaction mixture is 50 μ l including: 30 μ l of H₂O, 5 μ l of 10 x buffer (Buffer PB), 5 μ l of 10 x DNA, 5 μ l of 10 x human DNA polymerase beta, 5 μ l of 10 x dNTP mix. Incubate the reaction mixture in a standard black 96-well plate at 37°C for 60 min.



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Note: The final concentrations in the assay reaction are 30 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 1 mM DTT, 5 % glycerol, 150 nM DNA, 0.1 mM dATP, 0.1 mM dGTP. The recommended final enzyme concentration in the reaction mixture is 10 U/ml or 10 nM. The diluted enzyme should be prepared freshly.

3. Detection:

For each reaction, make 240 µl of detection solution by mixing 200 µl of Reagent U and 40 µl of the 1 x fluorescence dye. Mix 240 µl of detection solution with the 50 µl of reaction mixture. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Note: The fluorescence signal decreases in the first 30 min after addition of the detection solution. For quick tests, the fluorescence can be measured right after addition of the detection solution or at a fixed time point, for example, 5 min after addition of the detection solution. For HTS applications, where many plates may be accumulated and the reading time of different plates may vary, it is recommended reading the plates in 30 min or a longer period of time after addition of the detection solution.

Publications:

1. Merlo S., et al, Identification of 5-Methoxyflavone as a Novel DNA Polymerase-Beta Inhibitor and Neuroprotective Agent against Beta-Amyloid Toxicity. *J. Nat. Prod.* 78, 2704–2711 (2015).
2. Maldonado E. et al, Expression, purification, and biochemical characterization of recombinant DNA polymerase beta of the *Trypanosoma cruzi* TcI lineage: requirement of additional factors and detection of phosphorylation of the native form, *Parasitol Res* (2015) 114:1313–1326.

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
HDP A100K	Human DNA Polymerase Alpha Assay Kit
DPG100K	Human DNA Polymerase Gamma Assay Kit
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus

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