

**ProFoldin**

10 Technology Drive, Suite 40, Number 188

Hudson, MA 01749-2791 USA

Tel: (508) 735-2539

FAX: (508) 845-9258

www.profoldin.cominfo@profoldin.com

INSTRUCTIONS

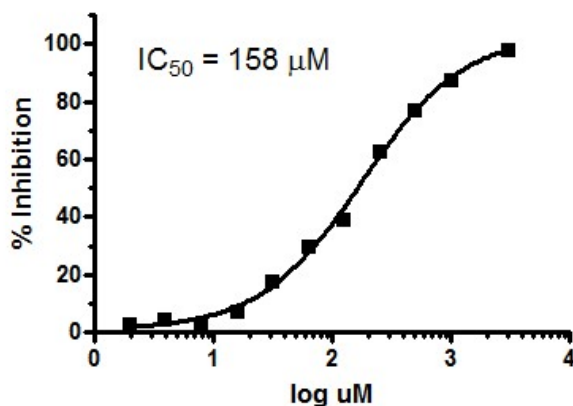
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E. coli DNA Polymerase III Alpha

E. coli* DNA Polymerase III Alpha – for 100 assays*Catalog No. DPA-100EC**

Protein construct:	Wild-type <i>E. coli</i> DNA Polymerase III Alpha purified from a bacterial expression system.
MW:	94 kDa
Enzyme concentration:	2 μ M
Enzyme activity assay:	The DNA polymerase activity is measured by using the DNA Polymerase III Alpha Assay Kit (Catalog No. DPA100K)
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.

***E. coli* polymerase III alpha
inhibited by ddGTP**



The *E. coli* DNA Polymerase III Alpha – for 100 assays (Catalog No. DPA-100EC) includes 33 μ l of 100 x *E. coli* DNA polymerase III alpha. It is for 100 assays.

Assay Protocol using the DNA Polymerase III Alpha Assay Kit

1. Reagent preparation:

10 x DNA: dilute the 100 x DNA with water.

10 x enzyme: prepare 100 x DNA polymerase using the 1 x buffer for dilution.

10 x dNTP mix: dilute the 100 x dNTP mix 10-fold with water.

1 x fluorescence dye: dilute the 10 x fluorescence dye 10-fold with water.



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2. Reaction:

The total volume of each reaction mixture is 30 µl including 18 µl of H₂O, 3 µl of 10 x Buffer DP, 3 µl of 10 x DNA, 3 µl of 10 x enzyme, 3 µl of 10 x dNTP mix. Incubate the reaction mixture at 37°C for 60 min.

Note: The final concentrations are 20 mM HEPES, pH 7.5, 10 mM Mg(OAc)₂, 1 mM DTT, 30 nM DNA, 20 nM DNA polymerase III alpha subunit, 0.1 mM dATP, 0.1 mM dGTP.

3. Detection:

Add 30 µl of the 1 x fluorescence dye into the 30 µl of the reaction mixture. Incubate 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:

DPA100K	DNA Polymerase III Alpha Assay Kit
DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
NLA100KE	<i>E. coli</i> NAD ⁺ -dependent DNA Ligase Assay Kit Plus
DNAB100KE	<i>E. coli</i> DNA Helicase ATPase assay Kit Plus-100
DNAB100KH	<i>H. influenzae</i> DNA Helicase ATPase assay Kit Plus-100
DNAB100KN	<i>S. pneumoniae</i> DNA Helicase ATPase assay Kit Plus-100
DNAB100KP	<i>P. aeruginosa</i> DNA Helicase ATPase assay Kit Plus-100
DNAB100KS	<i>S. aureus</i> DNA Helicase ATPase assay Kit Plus-100
EGA100KE	<i>E. coli</i> DNA Primase Assay Kit Plus
HGA100KE	<i>H. influenzae</i> DNA Primase Assay Kit Plus
PGA100KE	<i>S. pneumoniae</i> DNA Primase Assay Kit Plus
AGA100KE	<i>S. aureus</i> DNA Primase Assay Kit Plus
HDPA100K	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
MRPA100KE	Human Mitochondrial 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

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