



ProFoldin

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INSTRUCTIONS

ProFoldin DNA Polymerase Assay Kits

DNA Polymerase III Alpha Assay Kit

Catalog No. DPA100K

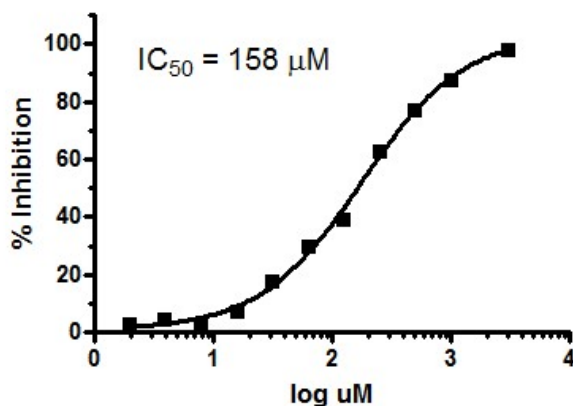
***E. coli* DNA Polymerase III Alpha Assay Kit Plus**

Catalog No. DPA100KE

Introduction

DNA polymerase III synthesizes DNA using the RNA primer made by the DNA primase at the DNA replication fork of bacteria. DNA polymerase III alpha is the catalytic subunit of the polymerase. The **DNA Polymerase Assay Kit** is based on measurement of the DNA molecules synthesized by the DNA polymerase. The assay is performed in a 384-well plate format. The assay can be used for detection of bacterial DNA polymerase III activity and high throughput screen of bacterial DNA polymerase inhibitors.

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



The **DNA Polymerase III Alpha Assay Kit** (Catalog No. DPA100K) includes 500 μ l of 10 x Buffer DP, 35 μ l of 100 x DNA template, 35 μ l of 100x dNTP mix and 320 μ l of 10x fluorescence dye for 100 assays of DNA polymerase reactions in a 384-well assay format. The assay conditions are optimized for bacterial DNA polymerase III alpha subunit. Enzyme is not included in the kit.

The ***E. coli* DNA Polymerase III Alpha Assay Kit Plus** (Catalog No. DPA100KE) includes all the reagents in the **DNA Polymerase III Alpha Assay Kit** (Catalog No. DPA100K) plus 35 μ l of 100 x *E. coli* DNA polymerase III alpha subunit.



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Assay Protocol

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.

2. Reaction:

The total volume of each reaction mixture is 30 μ l including 18 μ l of H₂O, 3 μ l of 10 x Buffer DP, 3 μ l of 10 x DNA, 3 μ l of 10 x enzyme, 3 μ l 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 μ l of the 1 x fluorescence dye into the 30 μ l 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

DPA100KH	<i>H. influenzae</i> DNA polymerase Assay Kit Plus
DPA100KN	<i>S. pneumoniae</i> DNA polymerase Assay Kit Plus
HDP A100KE	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
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Plus
HIV100KE	HIV Reverse Transcriptase Assay Plus

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