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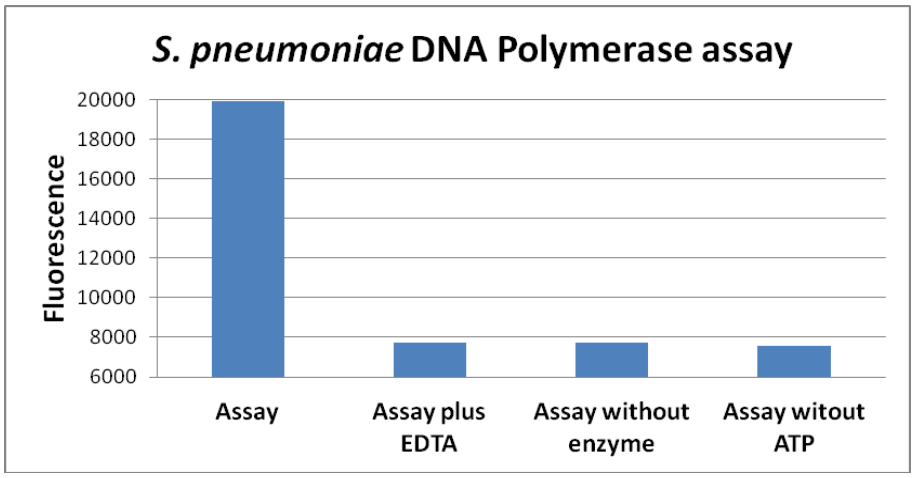
INSTRUCTIONS

ProFoldin
DNA Polymerase Assay Kits

DNA Polymerase III Alpha Assay Kit **Catalog No. DPA100K**
***S. pneumoniae* DNA Polymerase III Alpha Assay Kit Plus** **Catalog No. DPA100KN**

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.



The **DNA Polymerase III Alpha Assay Kit** (Catalog No. DPA100K) includes 500 µl of 10 x Buffer DP, 35 µl of 100 x DNA template, 35 µl of 100x dNTP mix and 320 µl 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 ***S. pneumoniae* DNA Polymerase III Alpha Assay Kit Plus** (Catalog No. DPA100KN) includes all the reagents in the **DNA Polymerase III Alpha Assay Kit** (Catalog No. DPA100K) plus 35 µl of 100 x *S. pneumoniae* 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 µl including 18 µl of H_2O, 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

Enzyme inhibition IC_{50} can be measured using the 384-well or 96-well plate assay format. Typically 50 x stock solutions with a 2-fold serial dilution in water or DMSO are prepared. For 96-well plate assays, 1.2 µl of the 50 x inhibitor is mixed with 53 µl of the assay reaction mixture composed of the buffer, DNA and enzyme for 5 min. Then 6 µl of 10 x NTP mix is added. At the end of the reaction, 60 µl of the 1x the dye is added and the fluorescence intensity is measured.

In order to accurately measure the IC_{50}, it is important to make sure the assay is in the linear range. The linear range can be defined by enzyme concentration-dependence and time-dependence experiments. The substrate concentrations should be optimized if needed.

Related products

E. coli DNA polymerase Assay Kit Plus	Catalog No.	DPA100KE
H. influenzae DNA polymerase Assay Kit Plus	Catalog No.	DPA100KH
Human DNA Polymerase Alpha Assay Kit	Catalog No.	HDPA100K
Human DNA Polymerase Beta Assay Kit	Catalog No.	DPB100K
Human DNA Polymerase Gamma Assay Kit	Catalog No.	DPG100K

More information of drug targets and enzyme assays

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