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INSTRUCTIONS

ProFoldin
DNA Polymerase Assay Kits

DNA Polymerase III Alpha Assay Kit **Catalog No. DPA100K**
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 can be performed in 96-well plate or 384-well plate format for high throughput screening of DNA polymerase inhibitors.

Each kit (Catalog number DPA100K) includes the assay buffer, DNA template, dNTP mix and fluorescence dye for 100 assays of DNA polymerase reactions in a 96-well plate format or 200 assays in a 384-well assay format. The assay conditions are optimized for bacterial DNA polymerase III alpha subunit. The following protocol is for the assays in 96-well plates. The kit DPA100K does not include DNA polymerase III alpha that can be purchased separately (please see the information of related products). The Kit DPA100KE includes the assay buffer, DNA template, dNTP mix, *E. coli* DNA polymerase III alpha and fluorescence dye.

Related Products:

DNAE-200EC	*E. coli* DNA polymerase III alpha subunit (for 200 assays)
DNAE-200HI	*H influenzae* DNA polymerase III alpha subunit (for 200 assays)
DPB100K	Human DNA polymerase beta assay kit (for 100 assays)

Assay Protocol

1. **Reagent preparation:**

   10 x DNA: dilute the 100 x DNA with water.
   1 x buffer: dilute the 10 x buffer (Buffer DP) 10-fold with water.
   10 x enzyme: prepare 100 nM DNA polymerase using the 1 x buffer for dilution.
   10 x dNTP mix: dilute the 100 x dNTP (10 mM dATP and dGTP) 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 40 µl including: 24 µl of H_2O, 4 µl of 10 x buffer (Buffer DP), 4 µl of 10 x DNA, 4 µl of 10 x enzyme, 4 µl of 10 x dNTP mix. Incubate the reaction mixture at room temperature for 60 min.

3. **Detection**:

   Add 80 µl of the 1 x fluorescence dye into the 40 µl of the reaction mixture. Incubate for 5 min. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.