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INSTRUCTIONS

ProFoldin DNA Polymerase III Alpha Assay Kit

Catalog Number **DPA100K**

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 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. Please adjust the reagent volumes accordingly for assays in 384-well plates.

Assay Protocol

1. Reagent preparation:

- 10 x DNA: dilute the 100 x DNA with water
- 10 x enzyme: prepare 100 nM DNA polymerase using the 1 x buffer for dilution
- 1 mM dNTP mix: a mixture of 1 mM dATP, 1 mM dGTP
- 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 μ l including: 24 μ l of H₂O, 4 μ l of 10 x buffer (Buffer DP), 4 μ l of 10 x DNA, 4 μ l of 10 x enzyme, 4 μ l of 1 mM dNTP mix. Incubate the reaction mixture at room temperature for 30 min.

3. Detection:

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

Note: Fluorescence signals are sensitive to temperature changes. Please keep the temperature consistent during the measurement.