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INSTRUCTIONS

ProFoldin RNA Polymerase Assay Kit

Catalog Number **RPA100K**

Introduction

The DNA-dependent RNA polymerase synthesizes RNA molecules using DNA as the template. The bacterial RNA polymerase is responsible for biosynthesis of mRNA, tRNA and rRNA in the cells. The protein is composed of 5 subunits including α , β , β' , ω , and σ with a MW \sim 400 kDa. The RNA Polymerase Assay Kit is based on measurement of the RNA molecules synthesized by the RNA polymerase. The assay can be performed in 96-well plate or 384-well plate format for high throughput screening of RNA polymerase inhibitors.

Each kit (Catalog number RPA100K) includes the assay buffer, DNA template and fluorescence dye for 100 assays of RNA polymerase reactions in a 96-well plate format or 200 assays in a 384-well assay format. The following protocol is for the assays in 96-well plates. Please adjust the reagent volumes accordingly for assays in 384-well plates. The assay buffer is optimized for *E. coli* RNA polymerase.

Assay Protocol

1. Reagent preparation:

- 10 x DNA: dilute the 100 x DNA with water
- 10 x enzyme: 100 nM RNA polymerase
- 1 mM NTP mix: a mixture of 1 mM ATP, 1 mM GTP, 1 mM CTP and 1 mM UTP
- 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 RP), 4 μ l of 10 x DNA template, 4 μ l of 10 x enzyme, 4 μ l of 1 mM NTP 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.