# DNA Polymerase I

# Instruction for Use

**Cat. No./Spec.: E1021-A/500 U, E1021-B/2,500 U**

**Concentration: 10 U/μL**

## Product Description

*This product is a template-dependent DNA polymerase capable of accelerating the synthesis of DNA in the 5′→3′ direction. It also possesses 3′→5′ exonuclease (proofreading) activity, 5′→3′ exonuclease activity, and RNase H activity.*

## Components

| *Component* | *E1021-A* | *E1021-B* |
|---|---|---|
| *DNA Polymerase I* | *50 μL* | *250 μL* |
| *10X Polymerase I Buffer* | *1 mL* | *1 mL × 5* |

## Storage Condition & Shelf Life

*Store at -20°C.*

## Source

*Recombinant E. coli strain containing the cloned gene polA from E. coli.*

## Unit Definition

*A unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of deoxynucleotides into a polynucleotide substrate within 30 minutes at 37° C.*

## Features

*- Incorporation of modified nucleotides (such as biotin, digoxigenin, or fluorescently labeled nucleotides).*

*- Compatible with a variety of buffer systems, including buffers for restriction enzymes, PCR, and RT reactions.*

## Scope of Application

*- Used in conjunction with DNase, for DNA labeling through nick translation.*

*- Used in conjunction with RNase H, for the synthesis of the second strand of cDNA.*

## Inhibition and Inactivation

*- Inhibitors: Metal chelating agents, PPi (inorganic pyrophosphate), Pi (inorganic phosphate) at high concentrations.*

*- Inactivation is achieved by heating at 75°C for 10 minutes or by the addition of EDTA.*

## Protocol

*Radioactive DNA labelling by nick-translation*

*① Prepare the reaction system:*

| *Component* | *Amount* |
|---|---|
| *10X Polymerase I Buffer* | *2.5 μL* |
| *Mixture of 3 dNTPs, 1 mM* each (without the labeled dNTP)* | *1.25 μL* |
| *[α-$^{32}$P]-dNTP, ~110 TBq/mmol (3000 Ci/mmol)* | *1.85-3.7 MBq (50-100 μCi)* |
| *DNase I, RNase-free (#E1018) freshly diluted to 0.002 U/μL*** | *1 μL* |
| *DNA Polymerase I* | *5-15 U* |
| *Template DNA* | *0.25 μg* |
| *Water, nuclease-free (#P9021)* | *To 25 μL* |

*Note:*

** To prepare a mixture of three unlabeled dNTPs (each at 1 mM), mix 1 μL of each dNTP sample (100 mM) with 97 μL of nuclease-free water. Store at -20°C.*

*** DNase I, RNase-Free can be diluted with the 1X reaction buffer of DNA Polymerase I.*

*② Incubate immediately at 15°C for 15-60 minutes.*

*③ Terminate the reaction by adding 1 μL of 0.5M EDTA, pH 8.0.*

*④ Take an aliquot (1 μL) to determine the efficiency of incorporation. The expected*

*specific activity of the DNA should be at least 108 cpm/μg.*

*⑤ If necessary, separate the labeled DNA from unincorporated radioactive precursors using a Sephadex G-50 or Bio-Gel P-60 chromatography column.*

**Notes**

- *DNA Polymerase I accepts modified nucleotides (such as biotin, digoxigenin, and fluorescently labeled nucleotides) as substrates for DNA synthesis.*
- *The reaction volume can be scaled up or down proportionally, provided that the final concentrations of the components (DNA, dNTPs, labeled dNTPs) are consistent with those shown in the protocol.*
- *Radioactively labeled DNA probes with higher specific activities can be prepared simultaneously using two radioactively labeled dNTPs. In this case, the composition of the unlabeled dNTP mixture should be adjusted accordingly.*

*This product is for research use only.*