Thermo SCIENTIFIC

PRODUCT INFORMATION

DNA Polymerase I

Pub. No. MAN0013723
Rev. Date 03 May 2016 (B.00)

Lot: _ **Expiry Date: _**

Components	#EP0041 500 U	#EP0042 2500 U
Concentration	10 U/µL	10 U/µL
10X Reaction Buffer	1 mL	5 x 1 mL

Store at -20 °C

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For Research Use Only. Not for use in diagnostic procedures.

Description

DNA Polymerase I, a template-dependent DNA polymerase, catalyzes 5'→3' synthesis of DNA. The enzyme also exhibits 3'→5' exonuclease (proofreading) activity, 5'→3' exonuclease activity and ribonuclease H activity.

Applications

- DNA labeling by nick-translation in conjunction with DNase I (1-3).
- Second-strand cDNA synthesis in conjunction with RNase H (4).

Source

E.coli cells with a cloned *polA* gene from *E.coli.*

Molecular Weight

103 kDa monomer.

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction 30 min at 37 °C.

Storage Buffer

The enzyme is supplied in: 25 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50% (v/v) glycerol.

10X Reaction Buffer

500 mM Tris-HCl (pH 7.5 at 25 °C), 100 mM $MgCl_2$, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: metal chelators, PP_i, P_i (at high concentrations) (5).
- Inactivated by heating at 75 °C for 10 min or by addition of EDTA.

Note

- DNA Polymerase I accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- Activity of DNA Polymerase I in Thermo Scientific buffers (in comparison to activity in assay buffer):

Buffers	Activity, %
for restriction enzymes: Thermo Scientific™ FastDigest™, FastDigest™ Green, O, R, 1X Thermo Scientific™ Tango™, 2X Tango™, BamHI, EcoRI	100
G	75-100
Ecl136II, PacI, SacI, KpnI	50-75
B	25-50
for *Taq* buffer with KCl, *Taq* buffer with $(NH_4)_2SO_4$ and *Pfu* buffer	100
for RT buffers	100

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

Incubation of supercoiled plasmid DNA with polymerase.

Quality authorized by:  Jurgita Zilinskiene

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Protocol for Radioactive DNA labelling by nick-translation

1. Mix the following components:

10X Reaction Buffer	2.5 µL
Mixture of 3 dNTPs, 1 mM* (without the labeled dNTP)	1.25 µL
[α-^{32}P]-dNTP ~110 TBq/mmol (3000 Ci/mmol)	1.85-3.7MBq (50-100 µCi)
DNase I, RNase-free (#EN0521) freshly diluted to 0.002 U/µL**	1 µL
DNA Polymerase I	0.5-1.5 µL (5-15 U)
Template DNA	0.25 µg
Water, nuclease-free (#R0581)	to 25 µL

***** To prepare the mixture of three non-labeled dNTPs (1 mM of each), mix 1 µL aliquots of stock solutions of each dNTP (100 mM, from #R0181) with 97 µL of Water, nuclease-free (#R0581). These dNTP mixes can be stored at -20 °C for further use.

****** DNase I, RNase-free (#EN0521) can be diluted with the 1X reaction buffer for DNA Polymerase I.

2. Immediately incubate at 15 °C for 15-60 min.
3. Terminate the reaction by adding 1 µL of 0.5 M EDTA, pH 8.0 (#R1021).
4. Take an aliquot (1 µL) to determine efficiency of the label incorporation. A specific activity of DNA at least 10^8 cpm/µg DNA is expected.
5. If needed, the labeled DNA may be separated from the unincorporated radioactive precursors on Sephadex G-50 or Bio-Gel P-60 column.

Note

- The reaction volumes can be scaled up or down providing that the final concentrations of the components (DNA, dNTPs, labeled dNTP) are as indicated in the protocol.
- Radioactive DNA probes with higher specific activities can be prepared using two radioactively labeled dNTPs simultaneously. In this case, the composition of the unlabeled dNTP mix should be adjusted accordingly.

References

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3. Yu, H., et al., Cyanine dye dUTP analogs for enzymatic labeling of DNA probes, Nucleic Acids Res., 22, 3226-3232, 1994.
4. Gubler, U., Hoffmann, B.J., A simple and very efficient method for generating cDNA libraries, Gene, 25, 263-269, 1983.
5. Eun, H-M., Enzymology Primer for Recombinant DNA Technology, Academic Press, INC, 1996.