

CERTIFICATE OF ANALYSIS

DNA Polymerase I, *E.coli*

#EP0042 2500u

Lot: **Expiry Date:**

Concentration: 10u/μl

Supplied with: 5x1ml of 10X Reaction Buffer

Store at -20°C

In total 6 vials.

Description

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

Source

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

Molecular Weight

103kDa monomer.

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30min at 37°C.

Enzyme activity is assayed in the following mixture: 67mM potassium phosphate (pH 7.4), 6.7mM MgCl₂, 1mM 2-mercaptoethanol, 0.033mM dATP, 0.033mM dTTP, 0.4MBq/ml [³H]-dTTP and 62.5μg/ml poly(dA-dT) poly(dA-dT).

Storage Buffer

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

10X Reaction Buffer

500mM Tris-HCl (pH 7.5 at 25°C), 100mM MgCl₂, 10mM DTT.

Applications

- DNA labeling by nick-translation in conjunction with DNase I (1-3), see protocol on back page.
Visit www.fermentas.com for protocol for Nick-translation using Biotin-11-dUTP.
- Second-strand synthesis of cDNA in conjunction with RNase H (4), see protocol at www.fermentas.com.

Inhibition and Inactivation

- Inhibitors: metal chelators, PP_i , P_i (at high concentrations) (5).
- Inactivated by heating at 75°C for 10min 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 Fermentas buffers (in comparison to activity in assay buffer):

Buffers	Activity, %
for restriction enzymes:	
O, R, 1X Tango™, 2X Tango™, BamHI, EcoRI	100
G	75-100
Ecl136II, SacI, KpnI	50-75
B	25-50
for <i>Taq</i> and <i>Pfu</i> DNA polymerases	100
for M-MuLV reverse transcriptases	100

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 20 units of DNA Polymerase I with 1µg of pBR322 DNA in 50µl of reaction buffer for 4 hours at 37°C.

Quality authorized by:

 Jurgita Zilinskiene

(continued on back page)

Protocol for DNA Labeling by Nick-translation

① Prepare the following reaction mixture:

10X reaction buffer	2.5µl
mixture of 3 dNTPs, 1mM* (without the labeled dNTP)	1.25µl
[α-³²P]-dNTP ~110TBq/mmol (3000Ci/mmol)	1.85-3.7MBq (50-100µCi)
Deoxyribonuclease I (DNase I), RNase-free (#EN0521) freshly diluted to 0.002u/µl**	1µl
DNA Polymerase I, <i>E.coli</i>	0.5-1.5µl (5-15u)
template DNA	0.25µg
Water, nuclease-free (#R0581)	to 25µl

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

** Deoxyribonuclease I (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 minutes.
- ③ Terminate the reaction by adding 1µl of 0.5M EDTA, pH 8.0 (#R1021).
- ④ Take an aliquot (1µl) to determine efficiency of the label incorporation. A specific activity of DNA at least 10⁸cpm/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.

DE-81 – Whatman anion exchange chromatography paper having diethylaminoethyl functional groups.

Whatman is a registered trademark of Whatman Ltd.

Sephadex is a registered trademark of Pharmacia Biotech, Inc.

Bio-Gel is a registered trademark of Bio-Rad Laboratories, Inc.

References

1. Ausubel, F.M., et al., Current Protocols in Molecular Biology, vol. 1, John Wiley & Sons, Inc., Brooklyn, New York, 3.5.3-3.5.6, 1994-2005.
2. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, Third Edition, Cold Spring Harbor laboratory, Cold Spring Harbor, N. Y., 2001.
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.

Related Products

- dNTP Set #R0181, #R0182, #R0186
- 10mM dNTP Mix #R0191, #R0192
- Aminoallyl-dUTP #R0091, #R1101
- Biotin-11-dUTP (not available in the USA) #R0081
- Fluorescein-12-dUTP (not available in the USA) #R0101
- dm⁶ATP #R0501
- dm⁴CTP #R0421
- dm⁵CTP #R0431
- M-MuLV Reverse Transcriptase #EP0351, #EP0352
- RevertAid™ M-MuLV Reverse Transcriptase (not available in the USA) #EP0441, #EP0442
- RevertAid™ H Minus M-MuLV Reverse Transcriptase (not available in the USA) #EP0451, #EP0452
- Ribonuclease H, *E.coli* #EN0201, #EN0202
- Deoxyribonuclease I (DNaseI), RNase-free #EN0521
- Pyrophosphatase, Inorganic (from yeast) #EF0221
- Water, nuclease-free #R0581, #R0582

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.