DNA Polymerase I (*E. coli*)

Catalogue number: MB42001, 500 U

Description

DNA Polymerase I (*E. coli*) is a non-thermostable DNA polymerase with inherent 3´→ 5´ (proofreading) and 5´→ 3´ exonuclease activities, in addition to a lower and non-specific ribonuclease H activity. The 5´→ 3´ exonuclease activity removes nucleotides ahead of the growing DNA chain, allowing nick-translation. Thus, DNA Polymerase I (*E. coli*) displays no strand-displacement activity and may be used for DNA labelling by nick-translation, in conjunction with DNase I, or second-strand cDNA synthesis, in conjunction with RNase H. DNA Polymerase I (*E. coli*) accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labelled nucleotides) as substrates for the DNA synthesis.

Storage conditions

DNA Polymerase I (*E. coli*) should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit of enzyme activity is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37 °C.

Enzyme concentration: 10 U/ µL

Inactivation

DNA Polymerase I (*E. coli*) is heat inactivated at 75 °C for 20 min.

System components and Reaction conditions

DNA Polymerase I (*E. coli*) is provided with a dedicated highly optimized NZYtech reaction buffer. The enzyme displays an optimum temperature of 37 °C, although it performs well at temperatures ranging from 15 °C – 37° C.

Protocol for DNA labelling by nick-translation

The following standard protocol serves as a general guideline for radioactive DNA labelling by nick-translation using DNA Polymerase I (*E. coli*). Preferably the enzyme should be added last.

1. Prepare the following 30 µL reaction:

Component	Volume
Substrate DNA	0.25 µg
DNA Polymerase I reaction buffer (10x)	3 µL
Mixture of 3 dNTPs (at 1 mM)*	1,5 µL
[α-^{32}P]-dNTP ~110 TBq/mmol (3000 Ci/mmol)	1.85-3.7MBq (50-100 µCi)
DNase I, RNase-free freshly diluted to 0.002 U/µL**	1 µL
DNA Polymerase I (*E. coli*)	1 µL (10 U)
Nuclease-free H_2O	up to 30 µL

** Prepare a mixture of three non-labelled dNTPs (1 mM of each).*
*** DNase I, RNase-free may be diluted with the 1x reaction buffer for DNA Polymerase I.*

2. Gently mix and pulse.

3. Incubate at 15 °C for 15-45 minutes.

4. Stop the reaction by adding 1 µL of 0.5 M EDTA, pH 8.0.

5. Take an aliquot (1 µL) to determine efficiency of the label incorporation (labelled DNA may be separated from the unincorporated radioactive precursors on Sephadex G-50 or Bio-Gel P-60 column).

Quality Control Assays

Purity

DNA Polymerase I (*E. coli*) is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYtech, Cat. No. MB15201).

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 10 U of DNA Polymerase I (*E. coli*) for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of DNA Polymerase I (*E. coli*) for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

DNA Polymerase I (*E. coli*) is assayed in a nick-translation reaction.

Related products:

Product name	Cat. No.
NZY DNase I	MB199
NZY RNase H (*E. coli*)	MB085
T4 DNA Polymerase	MB422
T4 DNA Ligase	MB007
dNTPs NZYSet	MB087
Water for Molecular Biology	MB111

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Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal Tel.: +351.213643514 Fax: +351.217151168
www.nzytech.com