For life science research only.
Not for use in diagnostic procedures.

Klenow Enzyme

Labeling grade
DNA polymerase I, large fragment, from *Escherichia coli* lysogenic NM 964

Cat. No. 11 008 404 001 100 U (2 U/ µl)
Cat. No. 11 008 412 001 500 U (2 U/ µl)

Version 13
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Store at −15 to −25°C

Product description

Storage buffer 50 mM potassium phosphate, 1 mM dithioerythritol, 50% glycerol (v/v), pH 7.0 (4°C).

Unit definiton One unit according to Richardson (+37°C, poly [d(A-T)] as primer) is the enzyme activity which incorporates 10 nmol of total nucleotides into an acid-precipitable fraction in 30 min under assay conditions (1).

Specific activity ≥ 5000 U/mg

Storage/stability The unopened reagent is stable at -15 to -25°C until the expiration date printed on the label.

Purity > 90% (SDS-PAGE) for up to 50 U of enzyme.

Incubation procedure 0.05-0.25 U of enzyme are incubated for 30 min at +37°C in a total volume of 300 µl incubation buffer.

Properties Klenow enzyme is the large fragment (M_r 75,000) of DNA polymerase I, and can be obtained by subtilisin treatment of the single polypeptide of DNA polymerase I (3). It carries the 5´→ 3´polymerase and the 3´→ 5´exonuclease activities of intact DNA polymerase I, but lacks the 5´→ 3´exonuclease activity of the native enzyme. The enzyme catalyzes the addition of mononucleotides from deoxynucleoside-5´-triphosphates to the 3´-hydroxyl terminus of a primer/template DNA. This property is used to synthesize DNA complementary to single-stranded DNA templates.

Typcal application

1). Use of Klenow for partial or complete filling of 3´recessed ends (after restriction enzyme digestion)

Protocol

Components	Reaction
Template DNA	1 µg DNA
Nucleotides*, final concentration	1 mM of desired dNTP* each
10× Filling buffer	2 µl
Klenow	1 U
H_2O	add up to 20 µl
Incubation	15 min at +37°C

* Please only add the desired dNTP´s as needed according to the sequence.

Filling buffer (10×) 500 mM Tris (pH 7.5), 100 mM $MgCl_2$, 10 mM DTT, 500 µg/ml BSA

Inactivation of enzyme Add 2 µl 0.2 M EDTA (pH 8.0) and/or heat to +65°C for 10 minutes.

2). Use of Klenow for random primed labeling

Protocol DNA is denatured by heating for 10 min at +95°C and **immediately** put on ice.

Components	Nonradioactive labels	Radioactive labels
Template DNA	10 ng - 3 µg DNA	10 ng - 2 µg DNA
Nucleotides, final concentration	100 µM of dATP, dCTP, dGTP each, 65 µM dTTP	25 µM of dATP, dCTP, dTTP each
Labeled nucleotides, final concentration	35 µM DIG-, Biotin-, or Fluorochrome-dUTP	[α^{32} P]dCTP 3,000 Ci/mmol), 50 µCi (1.85 MBq)
10× hexanucleotides mix (62.5 A_{260} U/ml)	2 µl	2 µl
Klenow enzyme	2 U	2 U
10× Labeling buffer	2 µl	2 µl
H_2O	add up to 20 µl	add up to 20 µl
Incubation	at least 60 min at +37°C	30 min at +37°C

Labeling buffer (10×) 500 mM Tris (pH 7.2), 100 mM $MgCl_2$, 1 mM DTT, 2 mg/ml BSA

Inactivation of enzyme Add 2 µl 0.2 M EDTA (pH 8.0) and/or heat to +65°C for 10 minutes.

Quality control

Lot-specific certificates of analysis are available at www.roche.com/certificates.

Incubation buffer for QC release 130 mM potassium phosphate, 6.5 mM $MgCl_2$, 33 µM dTTP, poly[d(A-T)], 0.833 A_{260} /ml, 0.068 µCi/ml U-^{14}C-dTTP, 1 mM dithioerythritol, 0.032 mg/ml bovine serum albumin, pH 7.4.

Absence of nicking activity 1 µg supercoiled pBR322 DNA is incubated for 16 h at +37°C with up to 50 U of Klenow enzyme in 50 µl buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH 7.5 at 37°C). No change in banding pattern is detectable.

Changes to previous version

- Editorial changes.

References

1 Richardson, C. C., et al. (1964) J. Biol. Chem. **239**, 222.
2 Laemmli, U. K. (1970) Nature **227,** 680.
3 Jacobsen, H. et al. (1974) Eur. J. Biochem. **45,** 623.
4 Telford, J. L. et al. (1979) Proc. Natl. Acad. Sci. USA **76,** 2590.
5 Feinberg, A. P., Vogelstein, B. (1983) Anal. Biochem. **132,** 6.

Ordering Information

Product	Application	Pack Size	Cat. No.
Deoxynucleoside Triphosphate Set	For many applications where high-quality nucleotides are required.	4 × 125 µmol (1,250 µl each) 4 × 25 µmol (250 µl each)	03 622 614 001 11 969 064 001
Water, PCR Grade	Specially purified, double-distilled, deionized, and autoclaved.	100 ml (4 vials of 25 ml) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001
Restriction Enzymes	DNA restriction digestion	Please refer to website or catalog.	
Rapid DNA Ligation Kit	Ligation of sticky-end or blunt-end DNA fragments in just 5 min at 15-25°C.	Kit (40 DNA ligations)	11 635 379 001
T4 DNA Ligase	Ligation of sticky- and blunt-ended DNA fragments.	100 U (1 U/µl) 500 U (1 U/µl)	10 481 220 001 10 716 359 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5´-phosphate residues from nucleic acids.	1,000 U (20 U/µl)	11 097 075 001
Agarose MP	Multipurpose agarose for analytical and preparative electrophoresis of nucleic acids.	100 g 500 g	11 388 983 001 11 388 991 001
Agarose Gel DNA Extraction Kit	For the elution of DNA fragments from agarose gels.	1 Kit (max. 100 reactions)	11 696 505 001
High Pure PCR Product Purification Kit	Purification of enzymatic modification reactions	1 kit (50 purifications) 1 kit (250 purifications)	11 732 668 001 11 732 676 001
Random Primed DNA Labeling Kit	Random-primed labeling of DNA (*e.g.,* plasmid or phage)	1 kit (50 labeling reactions)	11 004 760 001
DIG-High Prime DNA Labeling and Detection Starter Kit II	Nonradioactive random-primed labeling of DNA templates with DIG-11-dUTP.	1 kit (12 labeling reactions and 24 detection reactions)	11 585 614 910
DIG DNA Labeling Kit	Nonradioactive random-primed labeling of DNA templates with DIG-11-dUTP.	1 kit (40 labeling reactions)	11 175 033 910

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany