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Not for use in diagnostic procedures.



Klenow Enzyme

Labeling grade, from *Escherichia coli* lysogenic NM 964

 **Version: 14**
Content Version: June 2021

DNA polymerase I, large fragment

Cat. No. 11 008 404 001	100 U 2 U/ μ l
Cat. No. 11 008 412 001	500 U 2 U/ μ l

Store the product at -15 to -25°C .

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Klenow Enzyme, labeling grade	Solution (2 U/ μ l) in 50 mM potassium phosphate, 1 mM dithioerythritol, 50% glycerol (v/v), pH 7.0 at +4°C.	11 008 404 001	1 vial, 100 U
			11 008 412 001	1 vial, 500 U

1.2. Storage and Stability

Storage Conditions (Product)

When stored at -15 to -25°C , the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Klenow Enzyme, labeling grade	Store at -15 to -25°C .

1.3. Additional Equipment and Reagent required

For preparation of working solutions

See Section, **Working Solutions** for additional information on preparing solutions.

- Tris-HCl*
- MgCl_2 *
- DTT*
- BSA*

For filling of 3' recessed ends

- Water, PCR Grade*
- 0.2 M EDTA, pH 8.0

For random primed labeling

- Hexanucleotide Mix*
- Water, PCR Grade*
- Deoxynucleoside Triphosphate Set* or,
- dATP*, dCTP*, dGTP*, dTTP*
- Digoxigenin-11-dUTP*, or Biotin-16-dUTP*, or Fluorescein-12-dUTP*

1.4. Application

Use Klenow Enzyme to synthesize DNA complementary to single-stranded DNA templates.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Incubation procedure

0.05 to 0.25 U of enzyme are incubated for 30 minutes at +37°C in a total volume of 300 µl incubation buffer.

Working Solution

Solution	Composition	For use in...
Filling buffer, 10x conc.	500 mM Tris* (pH 7.5), 100 mM MgCl ₂ *, 10 mM DTT*, 500 µg/ml BSA*	Partial or entire filling of 3' recessed ends.
Labeling buffer, 10x conc.	500 mM Tris* (pH 7.2), 100 mM MgCl ₂ *, 1 mM DTT*, 2 mg/ml BSA*	Random primed labeling.

2.2. Protocols

Filling of 3' recessed ends

i See section, **Working Solution** for additional information on preparing solutions.

The following steps describe the filling of 3' recessed ends either partially or completely, after restriction enzyme digestion.

- 1 Prepare reaction mixture:

Reagent	Amount
Template DNA	1 µg
Nucleotides, final conc.	1 mM of desired dNTP ⁽¹⁾ each
Filling buffer, 10x conc.	2 µl
Klenow Enzyme	1 U
Water, PCR Grade*	add up to 20 µl
Total volume	20 µl

- 2 Incubate for 15 minutes at +37°C.
- 3 Inactivate enzyme by adding 2 µl 0.2 M EDTA, pH 8.0, and/or heat to +65°C for 10 minutes.

⁽¹⁾ Only add the desired dNTPs as needed according to the sequence.

Random primed labeling

i See section, **Working Solution** for additional information on preparing solutions.

1 Denature DNA by heating for 10 minutes at +95°C; immediately place on ice.

Reagent	Nonradioactive labels	Radioactive labels
Template DNA	10 ng – 3 µg DNA	10 ng – 2 µg DNA
Nucleotides, final conc.	100 µM of dATP*, dCTP*, dGTP* each, 65 µM dTTP*	25 µM of dATP*, dGTP*, dTTP* each
Labeled nucleotides, final conc.	35 µM Digoxigenin-11-dUTP*, Biotin- 16-dUTP*, or Fluorescein-12-dUTP*	[α ³² P]dCTP (3,000 Ci/mmol), 50 µCi (1.85 MBq)
Hexanucleotide Mix*, 10x conc. (62.5 A ₂₆₀ U/ml)	2 µl	2 µl
Klenow Enzyme	2 U	2 U
Labeling buffer, 10x conc.	2 µl	2 µl
Water, PCR Grade*	add up to 20 µl	add up to 20 µl
Total volume	20 µl	20 µl

2 For the nonradioactive labels, incubate for at least 60 minutes at +37°C.
– For the radioactive labels, incubate for 30 minutes at +37°C.

3 Inactivate enzyme by adding 2 µl 0.2 M EDTA, pH 8.0, and/or heat to +65°C for 10 minutes.

2.3. Parameters

Purity

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

Specific Activity

≥5,000 U/mg

Unit Definition

One unit (+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 minutes under assay conditions.

3. Additional Information on this Product

3.1. Test Principle

Properties

Klenow enzyme is the large fragment (M_r 75,000) of DNA polymerase I, obtained by subtilisin treatment of the single polypeptide of DNA polymerase I.

- 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.

3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols

 *Information Note: Additional information about the current topic or procedure.*

 **Important Note: Information critical to the success of the current procedure or use of the product.**

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.

Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001
MgCl ₂ Stock Solution	1 mL	11 600 770 103
	100 mL	11 600 788 103
1,4-Dithiothreitol (DTT)	custom fill	10 197 785 103
Bovine Serum Albumin, Molecular Biology Grade	custom fill	10 715 859 103
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
Hexanucleotide Mix	100 µl, 10x conc., 50 labeling reactions	11 277 081 001
dATP, PCR Grade	20 ml, 2,000 µmol	04 631 056 103
	100 ml, 10,000 µmol	11 889 516 103
dGTP, PCR Grade	20 ml, 2,000 µmol	04 631 129 103
	100 ml, 10,000 µmol	11 889 524 103
dCTP, PCR Grade	20 mL, 2,000 µmol	04 631 072 103
	100 mL, 10,000 µmol	11 889 508 103
dTTP, PCR Grade	20 mL, 2,000 µmol	04 631 137 103
	100 mL, 10,000 µmol	11 889 559 103
Deoxynucleoside Triphosphate Set	4 x 250 µl, 4 x 25 µmol, 100 mM	11 969 064 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM	03 622 614 001
Digoxigenin-11-dUTP, alkali-labile	25 nmol, 25 µl, 1 mM	11 573 152 910
	125 nmol, 125 µl, 1 mM	11 573 179 910
Biotin-16-dUTP	custom fill	11 093 711 103
Fluorescein-12-dUTP	custom fill	11 375 601 103

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

