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Not for use in diagnostic procedures.



Klenow Enzyme

Sequencing grade, from *Escherichia coli* lysogenic for NM 964

 **Version: 11**
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DNA polymerase I, large fragment

Cat. No. 10 104 531 001 250 U
 5 U/μl

Store the product at –15 to –25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Klenow Enzyme, Sequencing grade	Solution (5 U/μl) in 50 mM potassium phosphate, 1 mM dithioerythritol, 50% glycerol (v/v), pH 7.0 (+4°C).	1 vial, 250 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, Sequencing grade	Store at –15 to –25°C.

1.3. Additional Equipment and Reagent required

For preparation of incubation buffer

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

- MgCl₂*
- BSA*
- dATP*
- Dithioerythritol (DTE)
- Potassium phosphate

1.4. Application

Klenow enzyme is used to synthesize DNA complementary to single-stranded DNA templates in a wide variety of techniques:

- Conversion of 3'-recessed ends of restricted DNA fragments to blunt ends by fill-in.
- 3'-end labeling of DNA fragments using [$\alpha^{32}\text{P}$] deoxynucleotides.
- Second-strand synthesis of cDNA.
- DNA sequencing using the dideoxy chain terminator method.
- Elongation of oligonucleotides in site-directed mutagenesis.

2. How to Use this Product

2.1. Before you Begin

Working Solution

Incubation buffer

130 mM potassium phosphate, 6.5 mM MgCl₂*, 33 μM [¹⁴C]dTTP, poly[d(A-T)], 0.833 A₂₆₀ /ml, 33 μM dATP, 1 mM dithioerythritol, 0.032 mg/ml bovine serum albumin*, pH 7.4.

2.2. Protocols

Incubation procedure

Incubate 0.05 to 0.25 units of enzyme for 30 minutes at +37°C in a total volume of 300 μl Incubation buffer.

2.3. Parameters

Purity

3 μg of enzyme appear as a single band after electrophoresis in SDS polyacrylamide gels.

Volume Activity

5 U/μl

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.	
   etc.	Stages in a process that usually occur in the order listed.
   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		
Bovine Serum Albumin, Molecular Biology Grade	custom fill	10 715 859 103
dATP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 511 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 013 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM, 125,000 standard PCR assays of 20 µl each.	03 732 681 001
MgCl ₂ Stock Solution	3 x 1 ml	11 699 113 001

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.

