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Not for use in diagnostic procedures.



# DNA Polymerase I

## from *Escherichia coli* lysogenic for NM 964 (Kornberg polymerase) endonuclease-free

 **Version: 08**

Content Version: June 2021

Deoxynucleoside triphosphate: DNA deoxynucleotidyltransferase

**Cat. No. 10 642 711 001** 250 U

**Cat. No. 10 642 720 001** 1,000 U

**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	DNA Polymerase I	Solution (5 U/μl) in 50 mM potassium phosphate, 0.25 mM dithiothreitol, 50% glycerol (v/v); pH approximately 7.	10 642 711 001	1 vial 250 U
			10 642 720 001	1 vial 1,000 U

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at  $-15$  to  $-25^{\circ}\text{C}$ , the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	DNA Polymerase I	Store at $-15$ to $-25^{\circ}\text{C}$ .

## 1.3. Additional Equipment and Reagent required

### For determination of activity

- Potassium phosphate
- DTE
- $\text{MgCl}_2^*$
- dTTP\*
- dATP\*
- poly[d(A-T)]
- BSA (Bovine Serum Albumin)\*

### For nick translation

- Tris-HCl\*
- DTE
- $\text{MgCl}_2^*$
- Unlabeled dXTP
- [ $^3\text{H}$ ]-dTTP
- $\lambda\text{DNA}^*$
- DNase I\*

## 1.4. Application

Use DNA Polymerase I for:

- Analytical and preparative purposes.
- Syntheses which demand an endonuclease-free DNA polymerase.

## 2. How to Use this Product

### 2.1. Protocols

#### Determination of activity

- 1 Assay conditions are shown below:

Reagent	Concentration
Potassium phosphate, pH 7.4	130 mM
DTE	1 mM
MgCl <sub>2</sub>	6.5 mM
dTTP	33 μM
dATP (labeled plus unlabeled)	33 μM
Poly[d(A-T)]	0.25 A <sub>260</sub> U
Bovine Serum Albumin	10 μg
DNA Polymerase I	0.02 – 0.3 U
<b>Total volume</b>	<b>310 μl</b>

- 2 Incubate for 30 minutes at +37°C.

#### Nick translation assay conditions

- 1 Prepare the following mixture to check for the absence of endonucleases.

Reagent	Concentration
Tris-HCl, pH 7.5	50 mM
DTE	1 mM
MgCl <sub>2</sub>	10 mM
Unlabeled dXTP	20 μM each
[ <sup>3</sup> H]-dTTP, specific activity 90 – 110 Ci/mmol	50 μCi
λDNA	0.5 μg
DNase I	1.3 – 1.7 mU
DNA Polymerase I	1 U
<b>Total volume</b>	<b>20 μl</b>

- 2 Incubate for up to 120 minutes at +15°C.

## 2.2. Parameters

### Contaminants

The absence of any detectable endonuclease is tested by incubation of 10 U DNA Polymerase I with 1 µg pBR322 for 16 hours at +37°C. After electrophoresis, there is no detectable loss of the supercoiled form observed.

### EC-Number

EC 2.7.7.7

### Specific Activity

At least 8,000 U/mg (+37°C, poly[d(A-T)] as template).

### Unit Definition

One unit is the enzyme activity which produces in 30 minutes under assay conditions, the incorporation of 10 nmol of total nucleotide into an acid-precipitable fraction. This preparation shows one band after electrophoresis in SDS gradient polyacrylamide gels.

## 3. Supplementary Information

### 3.1. 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	
<b>i</b> Information Note: Additional information about the current topic or procedure.	
<b>⚠</b> 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.

### 3.2. Changes to previous version

Layout changes.  
Editorial changes.

### 3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001
Bovine Serum Albumin, Molecular Biology Grade	custom fill	10 715 859 103
dATP, PCR Grade	20 ml, 2,000 µmol	04 631 056 103
	100 ml, 10,000 µmol	11 889 516 103
dTTP, PCR Grade	20 mL, 2,000 µmol	04 631 137 103
	100 mL, 10,000 µmol	11 889 559 103
DNA, lambda	1 ml, 250 µg/ml	10 745 782 001
DNase I recombinant	2 x 10,000 U	04 536 282 001
MgCl <sub>2</sub> Stock Solution	3 x 1 ml	11 699 113 001

### 3.4. Trademarks

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

### 3.5. License Disclaimer

For patent license limitations for individual products please refer to:

**List of biochemical reagent products.**

### 3.6. Regulatory Disclaimer

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

### 3.7. Safety Data Sheet

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

### 3.8. 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.

