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

T7 RNA Polymerase
from *Escherichia coli* BL 21/pAR 1219

Version: 23
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Nucleoside-triphosphate: RNA nucleotidyltransferase (DNA-directed)

Cat. No. 10 881 767 001	1,000 U ≥ 20 U/µl
Cat. No. 10 881 775 001	5,000 U ≥ 20 U/µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	T7 RNA Polymerase	Enzyme storage buffer: 10 mM potassium phosphate, 200 mM KCl, 0.1 mM EDTA, 30 mM 2-mercaptoethanol, 50% glycerol (v/v), 0.1% Tween 20, pH 7.9 (+4°C).	10 881 767 001	1 vial, 1,000 U
			10 881 775 001	1 vial, 5,000 U
2	T7 RNA Polymerase, Transcription Buffer, 10x conc.	Transcription Buffer: 0.4 M Tris-HCl, pH 8.0 (+20°C), 60 mM $MgCl_2$, 100 mM dithiothreitol (DTT), 20 mM spermidine.	10 881 767 001	1 vial, 1 ml
			10 881 775 001	1 vial, 1 ml

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	T7 RNA Polymerase	Store at −15 to −25°C.
2	Transcription Buffer, 10x conc.	

1.3. Additional Equipment and Reagent required

For the radioactive assay

- Linear template DNA including T7 RNA promoter
- Ribonucleoside triphosphates*
- Labeled nucleotide [α-^{32}P] CTP [400 Ci/mmol; 15 TBq/mmol]
- RNase Inhibitor*
- Water, PCR Grade*
- 0.2 M EDTA, pH 8.0

For the nonradioactive assay

- Linear template DNA including T7 RNA promoter
- Ribonucleoside triphosphates*
- RNase Inhibitor*
- Water, PCR Grade*
- UTP
- Digoxigenin-11-UTP*, or
- Biotin-16-UTP*
- 0.2 M EDTA, pH 8.0

Alternatively, use 10x-concentrated RNA Labeling mixes that are specially designed for DIG- or biotin-labeling.*

For cold assay

- Linear template DNA including T7 RNA promoter
- Ribonucleoside triphosphates*
- RNase Inhibitor*
- Water, PCR Grade*
- 0.2 M EDTA, pH 8.0

For removal of unincorporated nucleotides

- Quick Spin Columns for radiolabeled DNA purification Sephadex G-50*

1.4. Application

T7 RNA Polymerase can be used in a variety of techniques:
- RNA or DNA blotting techniques.
- *In situ* hybridization
- Microarray target synthesis.
- Genomic sequencing
- For RNA sequencing, cloned DNA is transcribed with the enzyme in the presence of 3′-dNTPs. These nucleotides act as chain terminators in a similar manner to the ddNTP's in the Sanger sequencing method.
- RNase protection studies.
- Transcripts synthesized by the enzyme are used as precursor RNA for studies on RNA splicing and processing.
- It is also possible to synthesize capped RNA *in vitro* with addition of m7GpppG or m7GpppA in excess over GTP or ATP during the transcription reaction. The generated antisense RNA can be introduced into cells to suppress the expression of the corresponding genes.

2. How to Use this Product

2.1. Before you Begin

General Considerations

DNA removal

DNA can be removed with 20 U DNase I, RNase-free* and incubation for 15 minutes at +37°C.

Control of transcripts

Transcripts can be checked for length and integrity by native or denaturing gel electrophoresis.

Purification of transcript

Radioactively labeled transcripts can be purified from non-incorporated ribonucleoside triphosphates by column chromatography, for example, using Quick Spin Columns for radiolabeled DNA purification*, or from enzymatic components by phenol extraction.

⚠ ***Do not phenol/chloroform extract your DIG-labeled probe because it will partition into the organic phase.***

Labeling efficiency

The yield of the labeling reaction can be determined by trichloroacetic acid precipitation.

2.2. Protocols

Radioactive assay

1. Pipette the following components into a microfuge tube and mix.

Reagent	Amount	Final conc.
Template DNA	0.5 µg	–
Nucleotides ATP, GTP, CTP, UTP	–	0.5 mM final each
Labeled nucleotide [α-^{32}P] CTP [400 Ci/mmol; 15 TBq/mmol]	0.1 µl aqueous solution	–
Transcription Buffer, 10x conc. (Vial 2)	2 µl	–
T7 RNA Polymerase (Vial 1)	20 U	–
RNase Inhibitor*	20 U	–
Water, PCR Grade*	up to a final volume of 20 µl	–
Final Volume	**20**	

2. Incubate for 20 minutes at +37°C.

3. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0 and/or heat to +65°C.

Nonradioactive assay

1. Pipette the following components into a microfuge tube and mix.

Reagent	Amount	Final conc.
Template DNA	1 µg	–
Nucleotides ATP, GTP, CTP	–	1 mM final each
Labeled nucleotide: UTP, and DIG-11-UTP*, or Biotin-16-UTP*	–	 0.65 mM final 0.35 mM final
Transcription Buffer, 10x conc. (Vial 2)	2 µl	–
T7 RNA Polymerase (Vial 1)	40 U	–
RNase Inhibitor*	20 U	–
Water, PCR Grade*	up to a final volume of 20 µl	–
Final Volume	**20**	

2. Incubate for 2 hours at +37°C.

3. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0 and/or heat to +65°C.

Cold assay

1. Pipette the following components into a microfuge tube and mix.

Reagent	Amount	Fianl conc.
Template DNA	1 µg	–
Nucleotides ATP, GTP, CTP, UTP	–	1 mM final each
Transcription Buffer, 10x conc. (Vial 2)	2 µl	–
T7 RNA Polymerase (Vial 1)	40 U	–
RNase Inhibitor*	20 U	–
Water, PCR Grade*	up to a final volume of 20 µl	–
Final Volume	**20**	

2. Incubate for 2 hours at +37°C.

3. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0 and/or heat to +65°C.

2.3. Parameters

Activator

Strongly stimulated by BSA or spermidine.

Cofactors

T7 RNA Polymerase requires a DNA template and Mg^{2+} for the synthesis of RNA.

EC-Number

EC 2.7.7.6

Inhibition

Unlike bacterial RNA polymerases, T7 RNA Polymerase is not inhibited by the antibiotic rifampicin.

Molecular Weight

98 kDa (Single polypeptide chain)

Specificity

Promoter specificity

T7 RNA polymerase is extremely promoter-specific and only transcribes bacteriophage T7 DNA or DNA cloned downstream of a T7 promoter. Although the T7 and T3 promoter sequences differ by only 3 bp, T7 RNA Polymerase only transcribes DNA cloned downstream of its promoter.

Unit Definition

One unit is the enzyme activity which incorporates 1 nmol CMP in acid-precipitable RNA products within 60 minutes at +37°C.

Volume Activity

≥20 U/µl

3. Additional Information on this Product

3.1. Test Principle

T7 RNA polymerase is commonly used to transcribe DNA which has been cloned into vectors which have two phage promoters in opposite orientation. RNA can be selectively synthesized from either strand of the insert DNA with different polymerases. Homogeneously labeled single-stranded RNA can be generated with this system. Transcripts can be nonradioactively labeled with biotin* or DIG-11-UTP* or radioactively labeled to high specific activity with [α-^{32}P] or [α-^{35}S]-labeled nucleotides.

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.
❶ ❷ ❸ 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		
Protector RNase Inhibitor	2,000 U, 40 U/µl	03 335 399 001
	10,000 U, 5 x 2,000 U	03 335 402 001
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
Digoxigenin-11-UTP	250 nmol, 25 µl, 10 mM	11 209 256 910
	200 nmol, 57 µl, 3.5 mM	03 359 247 910
Biotin-16-UTP	250 nmol, 25 µl, 10 mM	11 388 908 910
Ribonucleoside Triphosphate Set	4 x 200 µl, 4 x 20 µmol, 100 mM each	11 277 057 001
DIG RNA Labeling Mix	40 µl, 20 transcriptions	11 277 073 910
DNase I recombinant, RNase-free	10,000 U, 10 U/µl	04 716 728 001
Quick Spin Columns for radiolabeled DNA purification	20 columns	11 273 965 001
	50 columns	11 273 973 001

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.

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