T7 RNA Polymerase
User Manual

Cat. No. **PC028 (5000U)**
Cat. No. **PC029 (25000U)**

User Manual

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T7 RNA Polymerase

I. Description

T7 RNA Polymerase is a DNA-dependent RNA polymerase with strict specificity for T7 promoter. The enzyme catalyzes the 5'→3' synthesis of RNA on either single-stranded DNA or double-stranded DNA downstream from the T7 promoter. T7 RNA Polymerase can enzymatically incorporates modified nucleotides (e.g., biotin-, digoxigenin-, fluorescein-labeled nucleotides) into RNA.

■ Advantages

✧ Capable of yielding up to 200 µg of RNA from a 1 µg template in a standard reaction.

✧ Requires only half the usual amount of enzyme in the standard reaction, while maintaining or potentially increasing yield.

✧ Enzymatically incorporates modified nucleotides modified NTP (e.g., aminoallyl-, biotin-, fluorescein-, digoxin-NTP).

■ T7 promoter sequence

5' - TAATACGACTCACTATAG - 3'

II. Contents and Storage

Store all components at -20℃ (stable for at least 12 months). Avoid repeated freezing/ thawing.

Cat. No.	Contents	Part No.	Quantity
PC028	T7 RNA Polymerase (50 U/µL)	PC028-01	100 µL
	10× Transcription Buffer	PC028-02	250 µL
PC029	T7 RNA Polymerase (50 U/µL)	PC028-01	100 µL×5
	10× Transcription Buffer	PC028-02	250 µL×5

■ Storage buffer

50 mM Tris-HCl, 100 mM NaCl, 20 mM β-ME, 1 mM EDTA, 50% Glycerol, 0.1% (w/v) Triton® X-100 (pH 7.9 @ 25℃)

■ Definition of activity unit

One unit is defined as the amount of enzyme that will incorporate 1 nmol ATP into acid-insoluble material in a total reaction volume of 50 µL in 1 hour at 37℃

III. Procedures

1. Prepare the reaction solution on ice according to the table below. Mix the reaction solution well. Spin down briefly.

Reagent	Volume	Final concentration
10× Transcription Buffer	2 μL	1×
RNA Inhibitor (40 U/μL)	0.5 μL	1 U/μL
NTP mix (25mM each)	8 μL	10 mM
DNA Template	1 μg	500 ng/μL
T7 RNA polymerse (50 U/μL)	1 μL	2.5 U/μL
DEPC-treated H2O	Up to 20 μL	

* RNaseLock® RNA Inhibitor (40 U/μL) Cat. No. PC005

2. Incubate at 37℃ for 2 h.
3. Optional: Use DNase I (Cat. No. PC024) to remove template DNA, following the protocol. To completely remove the template, it is recommended to do so after RNA purification.
4. Synthesized RNA can be purified by LiCl precipitation. LiCl precipitation of RNA is effective in removing the majority of unincorporated NTPs and enzymes.

■ **Note:**

a. When configuring the reaction, please add DEPC-treated water first. The enzyme and DNA template should be added last, and the reaction should be carried out immediately.
b. Linearized the DNA template with an enzyme that produces 5' protruding sticky ends or produces blunt ends, or by PCR. The amount of DNA template required is reduced when linearized by PCR.
c. Shorter DNA templates require longer reaction times. The reaction time can be adjusted according to the length of the DNA template (range 1 to 16 hours)
d. The greater the DNA template concentration, the faster the response. For the transcription of short template, should increase the template concentration.
e. When the DNA template concentration is high enough, continuing to increase the template concentration only affects the reaction speed, not the yield. Multiple reactions can be configured if you need to increase the yield.

- **FAQs:**

a. **Template for *in vitro* transcription with available T7 RNA polymerase?**

Double-stranded DNA, such as linear plasmids or PCR products.The DNA template needs to be linearized with an enzyme that produces 5' protruding sticky ends or blunt ends, or it can be linearized by PCR, with the amount of template required for the *in vitro* transcription reaction being reduced when linearised by PCR.

b. **Reasons for heterozygous bands in transcription products?**

First, make sure that the DNA is fully linearized as required and that the system is free from RNase contamination. If the IVT product is taken and directly subjected to non-denaturing electrophoresis, most of the spurious bands are formed due to the secondary structure of the RNA. In this case, denaturing gel electrophoresis is preferred. However, please do not denature the IVT product by heating before purification, as this may lead to RNA degradation. Also, there are a small number of unavoidable dsRNA by-products that can be inhibited by adding 1M urea to the reaction, or removed by subsequent purification.

IV. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of T7 RNA Polymerase (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged, or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product, or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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