Product Manual

Product Name	T7 RNA Polymerase（GMP-grade）
Source	Bovine Pancreas
Catalog Number	CSB-DEM078
Physical Form	Liquid
Enzyme activity	50U/μL
Storage Conditions	-20±5°C
Storage Buffer	20 mM sodium acetate (pH 6.5), 5 mM CaCl2, 0.1 mM PMSF, 50% glycerol
Activity definition	The enzyme amount required to incorporate 1 nmol of [3H] GMP into acid-insoluble precipitate within 1 hour at 37°C, pH 8.0, is defined as 1 unit.
Shelf Life	12 months

Product Description

This product is a recombinant T7 RNA polymerase expressed in Escherichia coli. It synthesizes RNA complementary to the downstream region of a double-stranded DNA template containing the T7 promoter sequence (5'-TAATACGACTCACTATAG*-3') using NTPs as substrates. Both linear blunt-ended or 5' overhang DNA can serve as substrates for T7 RNA polymerase, allowing linear plasmids and PCR products to be used as templates for in vitro RNA synthesis.

Product Components

Label	Components	Specifications		
1	T7 RNA Polymerase	5KU	25KU	50KU
2	10×Transcription Buffer	0.4mL	2mL	4mL

Operating instructions

1.The reaction system should be prepared according to the table below.

Components	Volume（μL）
10×Transcription Buffer	2
CTP / GTP/ ATP/ UTP (100 mM each)	0.4 each
T7 RNA Polymerase (50 U/μL)	0.5-1
RNase inhibitor (40 U/μL)	1

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RNase free H2O	Up to 18
Template DNA	2 (100 ng-1μg)

【Note】:a. Buffer and water should be equilibrated to room temperature before use.
b. The DNA template should be added last to prevent template precipitation.
c. If the transcript length is < 100 nt, the amount of template can be increased to 2 μg.

2.Incubate the reaction at 37°C for 1-2 hours (if the transcript length is ≤ 100 nt, increase the time to 4-8 hours).

3.After the reaction, add 2 U of DNase Ⅰ (RNase-free) at 37°C for 15 minutes to remove the DNA template.

4.Transcript Purification
In vitro transcribed products can be purified using RNA Cleaner magnetic beads to remove proteins, salt ions, and other impurities. Alternatively, a phenol/chloroform purification method can be employed.

Important Notes
- Recommended DNA templates: Linearized plasmids and PCR products containing the T7 promoter are recommended as templates.
- The purity of the transcription template significantly affects the in vitro transcription reaction. Residual RNase A from plasmid DNA extraction can greatly impact the quality of transcribed RNA. Phenol-chloroform extracted plasmid DNA is the preferred template. PCR products are recommended to be purified using gel recovery before use.
- For efficient transcription of specific regions, it is suggested to pre-cut the template DNA into blunt or 5' overhang ends downstream of the desired region.
- Adding 0.02 U of heat-stable inorganic pyrophosphatase to a 20 μL reaction system can significantly enhance transcription yield.