



## Product Information

### 9K-005-0004 (T7 RNA Polymerase (5000U))

**Description:** This product is bacteriophage T7 RNA polymerase recombinantly expressed in E. coli to contains the T7 promoter sequence (5'-TAATACGACTCACTATAG-3'). DNA is used as a template and NTP is used as a substrate to synthesize the reverse direction downstream of the promoter. Single stranded DNA complementary to RNA. Double-stranded linear blunt ends or 5' overhangs DNA can be used as a substrate template for T7 RNA polymerase, so linear plasmids, PCR products can be used as templates for in vitro synthesis of RNA.

#### Product Contents:

Components	9K-005-0004
T7 RNA Polymerase	5 KU (100 µl)
10X Transcription Buffer	400 µl

#### Transportation and storage methods:

Dry ice shipping. Store at -20°C, valid for two years.

#### Application:

1. Synthesize single-stranded RNA, including mRNA, siRNA, gRNA and other types of RNA precursor.
2. Synthesize labeled or unlabeled highly specific RNA probes.
3. Use cap analogs to synthesize capped mRNA.

#### Product Features:

Features	Description
Source	E. coli
Enzyme activity	≥ 50 U/µl
Unit Definition	Under the conditions of 37°C and pH8.0, 1 nmol can be produced within 1 hour. Enzyme required for incorporation of [3H] GMP into acid-insoluble precipitates. Amount is defined as 1 unit of activity.
Optimal reaction temperature	37°C



**Suggested reaction system and procedures:**

**1. Set up the following reaction system on the ice box:**

<b>Components</b>	<b>Dosage</b>	<b>Final Concentration</b>
10X Transcription Buffer	2 ul	1X
T7 RNA Polymerase	1ul	
ATP/CTP/GTP/UTP (100mM)	Each 0.4 $\mu$ l	Each 2mM
Template DNA	0.1 - 1 $\mu$ g	5 - 50 ng/ $\mu$ l
RNase Inhibitor (40 U/ $\mu$ l)	0.5 - 1 $\mu$ l	
RNase-free ddH <sub>2</sub> O	Up to 20 $\mu$ l	

- Gently mix the components with a pipette, collect by brief centrifugation, and incubate at 37°C for 3 hours. The reaction time can be adjusted appropriately according to the size of the product fragment. For example, if you synthesize RNA less than 0.3 kb, you can extend the reaction to 4 h or longer. A 16 h overnight reaction will not affect the quality of the product.
- After the reaction is completed, add 2  $\mu$ l of DNase I (RNase-free) to the reaction system, incubate at 37°C for 20 min to digest the transcribed DNA template, then add 2  $\mu$ l of 0.5 M EDTA and incubate at 65°C for 10 min to terminate the digestion reaction. (Optional)

**Notes:**

- It is recommended that template DNA be added to the reaction system last. Since 10X Transcription Buffer contains spermidine, too high a spermidine concentration will cause DNA precipitation.
- The purity of template DNA is crucial for in vitro transcription reactions. RNase A residues introduced during plasmid DNA extraction will significantly affect the quality of transcribed RNA. It is recommended to use high-purity RNase-free plasmids with an OD<sub>260/280</sub> of 1.8 - 2.0.
- Template DNA can be obtained by linearizing circular plasmid or PCR. Template DNA The upstream must contain T7 promoter sequence, and the downstream must be blunt-ended or protruding at the 5' end of the coding strand.
- Adding inorganic pyrophosphatase to the reaction system can significantly increase the transcription yield.