T7 RNA Polymerase

[Cat. No.] E-3043, E-3044

Introduction

T7 RNA Polymerase is a DNA-dependent RNA polymerase which initiates transcription highly specific on the T7 promoter. It is widely used for the rapid synthesis of specific RNAs *in vitro*.

Applications

- Synthesis of highly radiolabeled RNA probes
- Synthesis of siRNA precursors
- Synthesis of precursors for RNA splicing reactions
- Synthesis of mRNA for *in vitro* translation
- Synthesis of sgRNA for CRISPR-Cas9 based gene editing
- RNA structure, processing, and catalysis studies
- Production of RNA vaccines
- Expression control via anti-sense RNA

Components

Components	E-3043	E-3044
T7 RNA Polymerase (50 U/µl)	5,000 U (100 µl)	25,000 U (100 µl x 5)
5X Reaction buffer	1 ml	1 ml x 5
RNase inhibitor (100 ng/µl)	0.1 ml	0.1 ml x 5
100 mM DTT	0.2 ml	0.2 ml x 5

*** Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Specifications

T7 RNA Polymerase	
DNase activity	No
RNase activity	No
Protease activity	No

Buffer Composition

5X Reaction buffer	Contains 200 mM Tris-HCl, 30 mM $MgCl_2$, 10 mM Spermidine, pH 8.0

Storage Buffer

T7 RNA Polymerase is supplied in 50% (v/v) glycerol containing 20 mM sodium phosphate, 100 mM KCl, 0.5 mM EDTA, 1 mM DTT, and stabilizer, pH 7.7.

Unit Definition

One unit is defined as the enzyme activity which incorporates 1 nmole ATP into acid-insoluble products in 60 min at 37°C.

Quality Control

- Nuclease Contamination Assay: Nuclease activity is not detected after incubation of 1 µg of substrate Lambda DNA or RNA with 50 U of T7 RNA Polymerase at 37°C for 18 hrs.
- Protease Contamination Assay: Protease activity is not detected after incubation 2 µg of T7 RNA Polymerase at 37°C for 18 hrs.

Storage

Store at -20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label. To minimize degradation of DTT, store 100 mM DTT in small aliquots.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols.

Ordering Information

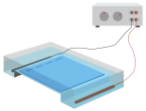
Description	Cat. No
T7 RNA Polymerase (5,000 U), 5X Reaction buffer, RNase inhibitor, 100 mM DTT	E-3043
T7 RNA Polymerase (25,000 U), 5X Reaction buffer, RNase inhibitor, 100 mM DTT	E-3044

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols

Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Thaw reagents</td><td>1. Thaw 5X Reaction buffer, 100 mM DTT, and RNase inhibitor on ice and mix thoroughly before use. Then, briefly spin down all components including linear template DNA, T7 RNA Polymerase, rNTPs (D-3003, not provided), and DEPC-D.W. (C-9030, not provided).</td></tr>
<tr><td>2</td><td> 
Preparation of reaction mixture</td><td>2. Add linear template DNA, 5X Reaction buffer, 100 mM DTT, rNTPs, T7 RNA Polymerase, RNase inhibitor and DEPC-D.W. into 0.2 ml PCR tubes (not provided) to a total volume of 20 µl.
• Preparation of reaction mixture
<table>
<tr><th>Components</th><th>20 µl reaction</th><th>Final concentration</th></tr>
<tr><td>Linear template DNA</td><td>Variable</td><td>0.2-1 µg</td></tr>
<tr><td>5X Reaction buffer</td><td>4 µl</td><td>1X</td></tr>
<tr><td>100 mM DTT</td><td>1 µl</td><td>5 mM</td></tr>
<tr><td>rNTPs (ATP, GTP, CTP, UTP)</td><td>Variable</td><td>Each 0.5 -1 mM</td></tr>
<tr><td>T7 RNA Polymerase (50 U/ µl)</td><td>1 µl</td><td>50 U</td></tr>
<tr><td>RNase inhibitor (100 ng/ µl)</td><td>0.5-1 µl</td><td>50-100 ng</td></tr>
<tr><td>DEPC-D.W.</td><td>Variable</td><td>-</td></tr>
<tr><td>Total volume</td><td>20 µl</td><td></td></tr>
</table>
* Note: For in vitro transcription, linearized plasmid DNA, PCR products, and synthetic DNA oligonucleotides can be used as template DNA.
3. Mix the reaction mixture by tapping or pipetting, and briefly spin down.</td></tr>
<tr><td>3</td><td> 
Incubate reactions in a thermal cycler</td><td>4. Incubate at 37°C for 1 hr. For shorter (< 300 nt) transcripts, incubate at 37°C for 2-16 hrs.</td></tr>
<tr><td>4</td><td> 
Analyze with gel electrophoresis</td><td>5. After the reaction, maintain the reaction mixture at 4-8°C. The samples can be stored at -20°C until use.
6. Mix the sample with RNA loading dye (not provided) and heat at 55°C for 15 min.
7. Load samples on denaturing agarose gel, and perform electrophoresis.</td></tr>
</table>