T7 RNA Polymerase

Instruction for Use

Cat. No./Spec.: E1022-A/5,000 U

Concentration: 20 U/μL

Product Description

Phage T7 RNA polymerase is a DNA-dependent RNA polymerase that has strict specificity for its corresponding double-stranded promoter. It catalyzes the synthesis of RNA in the 5'→3' direction downstream of the promoter, starting from the promoter on single-stranded DNA or double-stranded DNA.

Components

Component	*E1022-A*
T7 RNA Polymerase	*250 μL*
5X Transcription Buffer	*1.25 mL*

Storage Condition & Shelf Life

Store at -20°C.

Source

Recombinant E. coli strain containing the cloned gene encoding this enzyme.

Unit Definition

A unit is defined as the amount of enzyme required to incorporate 1 nmol of AMP into a polynucleotide substrate within 60 minutes at 37° C.

Features

- Capable of incorporating modified nucleotides (such as aminoallyl, biotin, fluorescein, digoxigenin-labeled nucleotides).

Scope of Application

The synthesis of unlabeled and labeled RNA can be used for:

• Hybridization, in vitro RNA translation

• As a substrate in aRNA, siRNA, RNase protection assays, and as a template for genomic DNA sequencing

• RNA splicing in studies of RNA secondary structure and RNA-protein interactions.

Inhibition and Inactivation

• Inhibitors: Metal chelating agents, enzyme activity is reduced by 50% when the concentration of NaCl or KCl is higher than 150 mM.

• Inactivation is achieved by heating at 70°C for 10 minutes or by the addition of EDTA.

Protocol

1. In Vitro Transcription

① Linearize the template DNA with a restriction endonuclease. Extract the DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve the DNA in DEPC-treated water (#R2041).

② Prepare the reaction system:

Component	*Amount*
5X Transcription Buffer	*10 μL*
ATP/GTP/CTP/UTP Mix, 10 mM each	*10 μL (2 mM final concentration)*
Linear template DNA	*1 μg*
RNase inhibitor (#R2011)	*50 U*
T7 RNA Polymerase	*30 U*
Depc-treated Water (#R2041)	*To 50 μL*

③ Incubate at 37°C for 2 hours.

④ Optional: To remove the template DNA, add 2 U of DNase I, RNase-Free (#E1018), mix, and incubate at 37°C for 15 minutes.

⑤ Inactivate DNase I by phenol/chloroform extraction.

Note:

- The transcription reaction should be performed under conditions that exclude RNase contamination. Pipettes, tubes, and water should be RNase-free. All solutions should be prepared in RNase-free water. It is recommended to wear gloves.

- The reaction mixture should be prepared at room temperature, as DNA may precipitate in the presence of spermidine at 4°C.

- Under the conditions described, more than 10 µg of RNA is obtained per 1 µg of template DNA.

- The yield of appropriately sized transcripts may be reduced if the template DNA is not fully linearized due to read-through reactions and the accumulation of longer transcripts with variable lengths.

- The reaction mixture can be scaled up or down proportionally.

Synthesis of radiolabeled RNA probes of high specific activity

① Linearize the template DNA with a restriction endonuclease. Extract the DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve the DNA in DEPC-treated water (#R2041).

② Prepare the reaction system:

Component	*Amount*
5X Transcription Buffer	*4 µL*
3 NTP mix, 10 mM each	*1 µL (0.5 mM final concentration)*
100 µM CTP	*2.4 µL (12 µM final concentration)*
[α-^{32}P]-CTP, ~30 TBq/mmol (800 Ci/mmol)	*1.85 MBq (50 µCi)*
Linear template DNA	*0.2-1.0 µg*
RNase inhibitor (#R2011)	*20 U*
T7 RNA Polymerase	*20 U*
Depc-treated Water (#R2041)	*To 20 µL*

③ Incubate at 37°C for 2 hours.

④ Terminate the reaction by cooling down to -20°C.

⑤ Determine the percentage of incorporation of the label into RNA.

Note:

- The RNA synthesized under the conditions described typically has a specific activity of 3-5x10^{8} dpm/µg.

- RNA can be radiolabeled with [^{32}P], [^{35}S], or [^{3}H]-labeled ribonucleotides. Use 1.85 MBq (50 µCi) of 5'-[α-^{32}P]-CTP with a specific activity of ~30 TBq/mmol (800 Ci/mmol), 11.1 MBq (300 µCi) of 5'-[α-^{35}S]-UTP with a specific activity of >37 TBq/mmol (>1000 Ci/mmol), and 0.925 MBq (25 µCi) of 5,6-[^{3}H]-UTP with a specific activity of 1.1-2.2 TBq/mmol (30-60 Ci/mmol) for a 20 µL reaction mixture.

- The yield of full-length transcripts decreases when the final concentration of the labeled NTPs is below 12 µM.

This product is for research use only.