Product Information

T7 RNA Polymerase from *Escherichia coli*

≥ 50,000 units/mL, aqueous solution

RT7POL

Product Description

The RNA Polymerase from phage T7 is a useful enzyme for *in vitro* RNA transcription. The T7 RNA polymerase is extremely promoter specific and transcribes only DNA downstream of a T7 promoter. The use of T7 RNA polymerase for *in vitro* transcription requires a DNA template with T7 promoter, millimolar concentrations of ribonucleoside triphosphates (rNTPs), and a specially formulated high-yield transcription buffer. Addition of Inorganic pyrophosphatase can enhance the reaction yield by preventing product inhibition of RNA polymerase, allowing the accumulation of large quantities of RNA. The DNA template can double stranded DNA either from a plasmid or PCR amplification.

This product is a recombinant protein expressed in *E. coli*. The product is supplied as an aqueous solution containing 50 mM Tris-HCl, 100 mM NaCl, 20 mM β-ME, 1 mM EDTA, 0.02% Tween® 20 (w/v) and 50% glycerol (w/v), pH 7.9 at 25 °C.

Unit definition

1 Unit of T7 RNA polymerase is defined as the amount of enzyme that synthesizes 1 µg of mRNA per hour in a 30-minute reaction containing 25 µg/mL linearized plasmid, 3 mM of each NTP and 18 mM $MgCl_2$, at 37 °C, pH 7.7.

Purity

Minimum ≥ 95% (by SDS-PAGE)

Activity

Minimum 50,000 units per mL

Nickase Activity

None detected in a 50 µL reaction containing 250 ng of supercoiled pUC19 DNA and at least 30 units of T7 RNA Polymerase incubated for 4 hours at 37 °C.

DNase Activity

None detected in a 50 µL reaction containing 250 ng linear pUC19 DNA and at least 30 units of T7 RNA Polymerase incubated for 16 hours at 37 °C.

RNase Activity

None detected in a 50 µL reaction containing 1.25-2.5 ng tRNA and at least 30 units of T7 RNA Polymerase is incubated for 4 hours at 37 °C.

Reaction Buffer

T7 RNA Polymerase is provided with RNA Polymerase buffer x10 (RPOLBUF) containing: 0.4 M Tris-HCl pH 8.0, 0.1 M $MgCl_2$, 20 mM Spermidine, 0.25 M NaCl, 50 mM DTT.

Example *in vitro* transcription protocol

Reaction Setup

Assemble the reaction mixture in the following order:

1. Add 10 µL of RPOLBUF (10X RNA Polymerase Buffer).
2. Add 5 µL of DTT 1 M, see Note 1 (next page).
3. Add 2.6 µL of $MgCl_2$ 1 M (Final concentration: 36 mM), see Note 2 (next page).
4. Add X µL of rNTPs (7.5 mM each, 30 mM total)
5. Add T7 transcription template, see Note 3 (next page).
6. (Optional) Add 2 µL of RNase Inhibitor 100 U/reaction.
7. (Optional) Add 2 µL of Inorganic pyrophosphatase 1 U/reaction.
8. Add 1 µL of T7 RNA Polymerase. The amount of T7 polymerase should be optimized. Use 100–1000 Units/reaction.
9. Adjust the final volume to 100 µL with H_2O.

Notes

1. Before opening enzyme vial spin down shortly.
2. Optional component. The final concentration of DTT can be increased up to 10 mM.
3. It is necessary to ensure that the $MgCl_2$ exceeds the total concentration of NTPs by 6 mM in the final reaction mixture.
4. The used template can be linearized plasmid, PCR amplified template or oligo template containing T7 promoter. The amount of the template should be optimized. A good starting point is to use 1-2 µg template in a 100 µL reaction.

Incubation

Incubate the reaction at 37 °C for 1-8 hours.

DNase Treatment

1. Add 5 µL of DNase (1 unit/µL, RNase-free).
2. Incubate at 37 °C for 30 minutes.

Purification

Purify the RNA products using a desalting gel filtration column. If necessary, further purify using a preparative gel.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store this product at –20 °C (range of –25 °C to –10 °C). The product retains activity for at least 2 years when stored at –20 °C.

Reagents (Not supplied)

Product Name	Catalog Number
Inorganic pyrophosphatase	SAE0215
RNase Inhibitor	R1158
Nucleotides	
CTP	C8552
ATP	A6559
GTP	G3776
UTP	U1006
$MgCl_2$	M1028
DTT	D9779
Water	W4502
DNase I, RNase free	AMPD1, D2821 or D5319
S-(5′-Adenosyl)-L-methionine chloride dihydrochloride	A7007
KOD One™ PCR Master Mix	KMM-101NV

References

1. Rong M. "Promoter specificity determinants of T7 RNA polymerase" Vol 95(2) :515-519. 1998.
2. Timothy W. Nilsen, Donald C. Rio, and Manuel Ares Jr. "High-Yield Synthesis of RNA Using T7 RNA Polymerase and Plasmid DNA or Oligonucleotide Templates": March 24, 2024.

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