

Hieff™ T7 RNA Polymerase GMP-grade(low dsRNA, 250 U/μL)

Product description

This product is a bacteriophage T7 RNA polymerase derived from recombinant expression in *E. coli*. It is a T7 RNA polymerase obtained by mutation after modification and optimization of wild-type T7, which can significantly reduce the dsRNA content. Double-stranded DNA containing the T7 promoter sequence is used as a template and NTP is used as a substrate to synthesize RNA complementary to the reverse single-stranded DNA downstream of the promoter. Double-stranded linear blunt-end or 5' protruding end DNA can be used as a substrate template for T7 RNA polymerase, so linear plasmids and PCR products can be used as templates for in vitro RNA synthesis.

Specifications

Cat.No.	10629ES10 / 10629ES60 / 10629ES72 / 10629ES86 / 10629ES96 / 10629ES99
Size	10 KU / 100 KU / 250 KU / 2500 KU / 25 MU / 100 MU

Components

Name	10629ES10 (10 KU)	10629ES60 (100 KU)	10629ES72 (250 KU)	10629ES86 (2500 KU)	10629ES96 (25 MU)	10629ES99 (100 MU)
T7 RNA Polymerase (low dsRNA, 250 U/μL)	40 μL	400 μL	1 mL	10 mL	100 mL	400 mL

[Note]: T7 RNA Polymerase can be used in combination with 10× Transcription Buffer 3 (Cat#10674).

Product Properties

Source	Recombinant <i>E. coli</i>
Optimum temperature	37°C
Activity	250 U/μL
Residual host nucleic acid	<10 fg/U
Residual host protein	<50 ppm
Residual RNase	Negative
Storage buffer	50 mM Tris-HCl, 1 mM EDTA, 10 mM DTT, 100 mM NaCl, 0.1% Triton X-100, 50% (v/v) glycerol, pH7.9@25°C
Activity unit definition	The amount of enzyme required to incorporate 1 nmol of [3H] GMP into the acid-insoluble precipitate within 1 hour at 37°C and pH8.0 is defined as one activity unit.

Storage

This product should be stored at -25~-15°C for 1 year.

Instructions

1. Take out the experimental materials from -20°C, thaw them on an ice box, mix them thoroughly, centrifuge briefly to collect the liquid at the bottom of the tube, and place them on ice for later use.

2. Preparation of reaction system:

2.1 General transcription system

Components	Volume(μL)	Final Concentration
10×Transcription Buffer	2	1×
CTP/GTP/ATP/UTP (100 mM each)	2 each	10 mM each
T7 RNA Polymerase (low dsRNA, 250 U/μL)	1-1.6	-
Murine RNase inhibitor (40 U/μL)	0.5	-
Pyrophosphatase, Inorganic (0.1 U/μL)	0.4	-
template DNA	X (1 μg)	-
RNase free H ₂ O	Up to 20	-

2.2 Co-transcriptional capping system

If co-transcriptional capping is selected, prepare the co-transcriptional capping system according to the following table:

Components	Volume(μL)	Final Concentration
10×Transcription Buffer	2	1×
CTP/GTP/ATP/UTP (100 mM each)	2 each	10 mM each
Cap1 Analog (100 mM)	2	10 mM
T7 RNA Polymerase (low dsRNA, 250 U/μL)	1-1.6	-
Murine RNase inhibitor (40 U/μL)	0.5	-
Pyrophosphatase, Inorganic (0.1 U/μL)	0.4	-
template DNA	X (1 μg)	-
RNase free H ₂ O	Up to 20	-

[Note]:

- 1) If the transcription length is < 100 nt, the template input can be increased to 2 μg and the transcription time can be increased to 4-8 h.
- 2) In order to improve the quality of RNA, it is recommended to use a plasmid linearized template for transcription.
- 3) The above reaction system is the recommended reaction. NTP, cap addition amount, T7 RNA polymerase, etc. can be adjusted appropriately according to the actual situation.
- 4) 20 μL transcription system, referring to the above system, can obtain 180-240μg of RNA. If you want to obtain more RNA, you can scale up the reaction system in equal proportion without affecting the reaction.
- 5) Use reagents, containers, etc. without RNase contamination.

3. React at 37°C for 2-3 h.

4. After the reaction, add 1 μL DNase I (Cat #10611ES) and react at 37°C for 15-30 min to remove the DNA template.

5. The synthesized RNA needs to be purified and quality controlled later. The qualified samples can be used for subsequent experiments.

Notes

1. This product is for research use only.
2. Please operate with lab coats and disposable gloves, for your safety.