KACTUS

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Premium T7 RNA Polymerase, GMP-Grade

Catalog #GMP-T7P-EE1MP

Form: Liquid
Source: *E. coli*
Production Requirement: Clean (Grade C or D)
Product Grade: GMP
Storage Buffer: 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 2 mM DTT, 0.1% Triton X-100, 50% Glycerol, pH 7.9
Concentration: 200U/μL
Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1nmol ATP into acid-soluble material in a total reaction volume of 50μL in 1 hour at 37°C.
Storage and stability: -20°C ± 5°C for 24 months. Avoid repeated freeze/thaw cycles.

Product Description

Premium T7 RNA Polymerase is an engineered T7 RNA polymerase. By mutating critical amino acids in the wild-type sequence, the premium T7 RNA polymerase can efficiently reduce the generation of dsRNA byproducts. The premium T7 RNA polymerase uses single or double-strand DNAs harboring T7 promoter as templates to synthesize complementary RNAs in the presence of NTPs.

This product is produced in accordance with GMP requirements using the company's SAMS™ protein production platform optimized by the E. coli expression system and purification process.

Applications

- mRNA for *in vitro* translation
- Radiolabeled RNA probe
- Non-isotopic RNA labeling
- RNA vaccines preparation
- Guide RNA preparation
- Antisense RNA preparation
- Capped mRNA synthesis with cap analog

Quality Control Statement

This product has been filed with the FDA Drug Master Files and is assigned DMF #037660. KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. The production is antibiotic- and animal-free.

Quality Control Release Criteria

Assay	Criteria
Activity (Probe Incorporation)	200.0-320.0kU/ml
Purity (SEC-HPLC)	≥ 95%
Concnetration	3.2mg/ml±20%
Residual Nickel Salt	≤ 10 ppm
Endotoxin	≤ 10EU/ml
Residual DNase	Negative
Residual RNase	Negative
Residual Protease	Negative
Residual Host Cell Protein	≤ 20ng/mg
Residual Nickel Salt	≤ 10.0ppm
Residual Heavy Metal	≤ 10.0ppm
Bioburden	≤ 1 CFU/10mL

I. Protocol for *In Vitro* Transcription

1) Prepare the following reaction mixture.

Reagent	Quantity
5X Transcription Buffer 1 or 2	4μL
CTP/GTP/ATP/UTP (100mM each)	1.5 μL each
Murine RNase Inhibitor (120U/μL)	0.5μL
Pyrophosphatase, Inorganic (0.1U/μL)	1μL
Premium T7 RNA Polymerase(200 U/μl)	0.5μL
Template DNA	1μg
RNase-free Water	Up to 20μL

2) Incubate at 37°C for 1-2 hours.
3) After transcription, add 2U DNase I to remove DNA template for 15 minutes at 37°C.
4) Inactivate DNase I by phenol/chloroform extraction.

Note: The reaction product can be very sticky. DNase I is recommended to dilute the reaction mixture.

II. **Protocol for Co-transcription IVT with Capping analog**

1) Prepare the following reaction mixture.

Reagent	Quantity
5X Transcription Buffer 1 or 2	4μL
CTP/GTP/ATP/UTP (100mM each)	1.5 μL each
CAP1-Analog(100 mM)	1.2μL
Murine RNase Inhibitor (120U/μL)	0.5μL
Pyrophosphatase, Inorganic (0.1U/μL)	1μL
Premium T7 RNA Polymerase(200 U/μl)	0.8μL
Template DNA	1μg
RNase-free Water	Up to 20μL

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Note：5×Transcription Buffer 1 is suitable for sequence length <3000 nt with higher yieldBuffer 2 is used for synthesizing mRNAs >3000 nt for better product integrity. Please choose the better buffer option based on actual applications.

2) Incubate at 37℃ for 1-2 hours（If the transcript length is ≤100 nt, extend the reaction time to 4-8 hours）。
3) Use 2 U DNase I to remove the DNA template. Incubate at 37℃ for 15 min。

Note：The reaction product can be very sticky. DNase I is recommended to dilute the reaction mixture.

Notes

- Murine RNase Inhibitor is added to protect RNA from possible RNase contamination.
- The reaction mixture should be prepared at room temperature as DNA may precipitate in the presence of spermidine at 4°C.
- The reaction may be scaled up as needed.