## MaxPure™ 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, 10 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:**  $-20^{\circ}C \pm 5^{\circ}C$  for 24 months. Avoid repeated freeze/thaw cycles.

#### **Product Description**

MaxPure<sup>™</sup> T7 RNA Polymerase is an engineered T7 RNA polymerase. By mutating critical amino acids in the wild-type sequence, the MaxPure<sup>™</sup> T7 RNA polymerase can efficiently reduce the generation of dsRNA byproducts. The MaxPure<sup>™</sup> 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<sup>™</sup> 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%	
Concentration	3.2mg/ml±20%	
Residual Nickel Salt	≤ 10 ppm	
Endotoxin	≤ 10EU/mI	
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
MaxPure™ T7 RNA Polymerase(200 U/µI)	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 <u>DNase I</u> 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
MaxPure™ T7 RNA Polymerase(200 U/μI)	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.

- Incubate at 37°C for 1-2 hours (If the transcript length is ≤100 nt, extend the reaction time to 4-8 hours)₀
- 3) Use 2 U DNase I to remove the DNA template. Incubate at 37  $^\circ C$  for 15 min $_\circ$

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.