

# MaxPure™ T7 RNA Polymerase

## Product Information

Product Name	Catalog-SKU	Size
MaxPure™ T7 RNA Polymerase	T7P-EE1MP-A	20 KU
	T7P-EE1MP-B	100 KU
	T7P-EE1MP-C	200 KU

## Product Description

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

## Product Specification

Component	T7P-EE1MP-A (20 kU)	T7P-EE1MP-B (100 kU)	T7P-EE1MP-C (200 kU)
T7 RNA Polymerase V2.0 (200 U/μl)	T7P-EE1MP-A1 (100 μl)	T7P-EE1MP-B1 (500 μl)	T7P-EE1MP-C1 (1 ml)
5×Transcription Buffer 1(for <3000 nt)	T7P-EE1MP-A2 (1.5 ml)	T7P-EE1MP-B2 (7.5 ml)	T7P-EE1MP-C2 (15 ml)
5×Transcription Buffer 2(for >3000 nt)	T7P-EE1MP-A3 (1.5 ml)	T7P-EE1MP-B3 (7.5 ml)	T7P-EE1MP-C3 (15 ml)

**Source** *E.coli*

**Storage Buffer** 50 mM Tris-HCl, 100 mM NaCl, 10mM DTT, 1 mM EDTA, 50% Glycerol, 0.1% Triton X-100, pH 7.9

**Enzyme Activity Unit Definition** The amount of enzyme that will incorporate 1 nmol of ATP into acid-precipitable material in 1 hour at 37°C.

**Transportation/Storage** Ship on dry ice. Store at -20 ± 5°C. Avoid repeated freeze-thaw cycles

## Application

RNA *In Vitro* transcription (IVT) Capped mRNA synthesis

## Protocol

### IVT

(1) Prepare the reaction mix at room temperature :

Component	Volume
RNase-free Water	To 20 $\mu$ l
5 $\times$ Transcription Buffer 1 or 2	4 $\mu$ l
CTP/GTP/ATP/UTP or N1-Me-Pseudo UTP (100 mM each)	1.5 $\mu$ l each
Murine RNase Inhibitor(120 U/ $\mu$ l)	0.5 $\mu$ l
Pyrophosphatase, Inorganic(0.1 U/ $\mu$ l)	1 $\mu$ l
DNA	To 1 $\mu$ g
T7 RNA Polymerase V2.0(200 U/ $\mu$ l)	0.5 $\mu$ l

Note : 5 $\times$ Transcription Buffer 1 is suitable for sequence length <3000 nt with higher yield Buffer 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°C for 1-2 hours (If the transcript length is  $\leq$ 100 nt, extend the reaction time to 4-8 hours) 。

(3) Use 2 U DNase I to remove the DNA template. Incubate at 37°C for 15 min. 。

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

### Co-transcription IVT with Capping analog

(1) Prepare the reaction mix at room temperature :

Component	Volume
RNase-free Water	To 20 $\mu$ l
5 $\times$ Transcription Buffer 1 or 2	4 $\mu$ l
CTP/GTP/ATP/ UTP or N1-Me-Pseudo UTP(100 mM each)	1.5 $\mu$ l each
CAP1-Analog(100 mM)	1.2 $\mu$ l
Murine RNase Inhibitor(120 U/ $\mu$ l)	0.5 $\mu$ l
Pyrophosphatase, Inorganic(0.1 U/ $\mu$ l)	1 $\mu$ l
DNA	To 1 $\mu$ g
T7 RNA Polymerase V2.0(200 U/ $\mu$ l)	0.8 $\mu$ l

Note : 5 $\times$ Transcription Buffer 1 is suitable for sequence length <3000 nt with higher yield Buffer 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°C for 1-2 hours (If the transcript length is  $\leq$ 100 nt, extend the reaction time to 4-8 hours) 。

(3) Use 2 U DNase I to remove the DNA template. Incubate at 37°C for 15 min. 。

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

## Assay Data

The MaxPure™ T7 RNA Polymerase significantly reduced the amount of dsRNA byproducts.

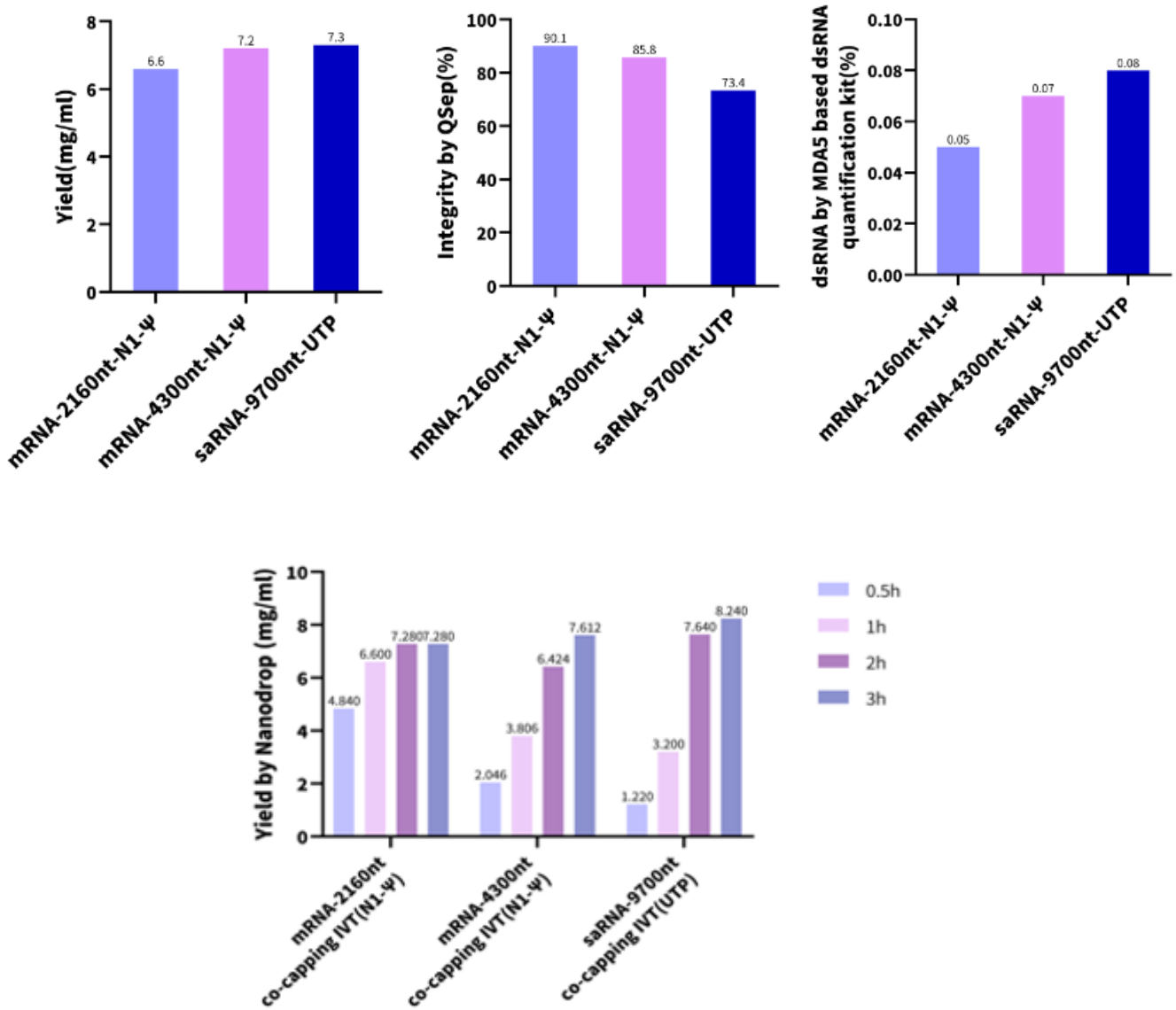


Fig. The result shows that MaxPure™ T7 RNA Polymerase can effectively reduce the level of dsRNA without affecting the quantity and quality of mRNA products.

## Cautions

- (1) To avoid the interference of residual protein and ions, the linearized plasmids need to be purified before IVT
- (2) DNA templated needs to be cut as blunt or 5' stick end for higher transcription efficiency in certain specific regions.
- (3) For research use only. Not for other purposes.