Premium T7 RNA Polymerase

Product Information

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

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.

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, 2mM 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℃. Avoid repeated freeze-thaw cycles

Application

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

tel：(+1) 617-665-7333
website：www.kactusbio.com

Protocol

IVT

（1）Prepare the reaction mix at room temperature：

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

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. 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 µl
5×Transcription Buffer 1 or 2	4 µl
CTP/GTP/ATP/ UTP or N1-Me-Pseudo UTP(100 mM each)	1.5 µl each
CAP1-Analog(100 mM)	1.2 µl
Murine RNase Inhibitor(120 U/µl)	0.5 µl
Pyrophosphatase, Inorganic(0.1 U/µl)	1 µl
DNA	To 1 µg
T7 RNA Polymerase V2.0(200 U/µl)	0.8 µl

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 viscous. DNase I is recommended to dilute the reaction mixture.**

tel：(+1) 617-665-7333
website：www.kactusbio.com

Assay Data

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

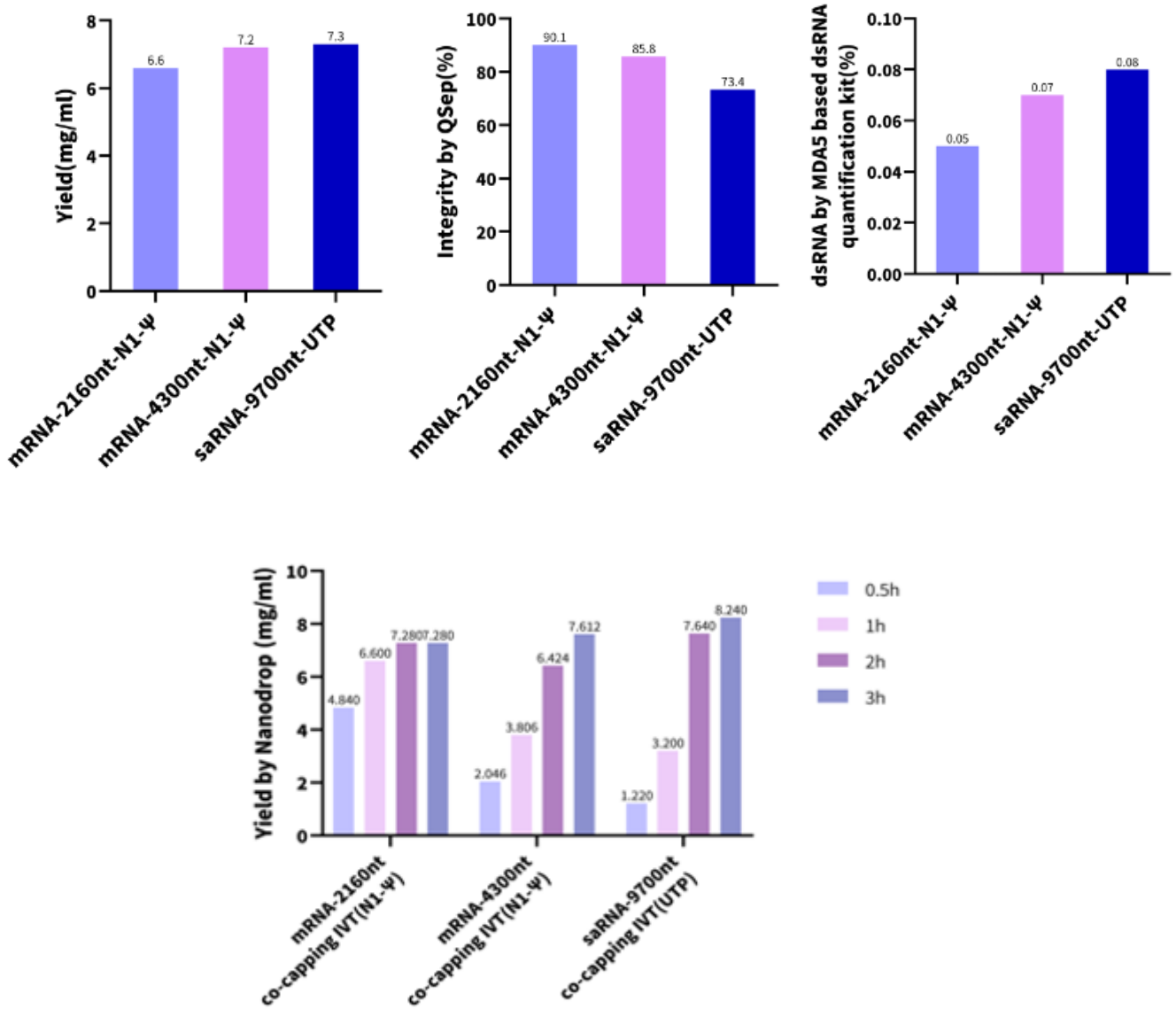

Fig. The result shows that Premium T7 RNA Polymerase can effectively reduce the level of dsRNA without affecting the quantity and quality of mRNA products.

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.

tel：(+1) 617-665-7333
website：www.kactusbio.com