T7 RNA polymerase (low dsRNA)

ARETERNA
A Synthgene Company

PRODUCT DESCRIPTION

This enzyme is a mutant form of T7 RNA polymerase derived from *E. coli* expression intended for use in in-vitro transcription (IVT) to generate mRNA. It utilizes double-stranded DNA with the T7 promoter sequence (5'-TAATACGACTCACTATA-3') as a template and NTPs or modified NTPs as substrate. Either linearized plasmids or PCR products can be used as IVT templates. Compared to the wild-type T7 RNA polymerase, this product generates much lower level of double-strand RNA (dsRNA) during the IVT process, which makes the mRNA less immunogenic.

Product Name	Size	Cat. No.
T7 RNA polymerase (low dsRNA)	100 µL/20KU	10308-20K
	1 mL/200KU	10308-200K
	10 mL/2MU	10308-2M

Note: the product comes with a 5x Transcription Buffer (low dsRNA)

FEATURES AND BENEFITS

- **Lower dsRNA from IVT**
- **Reduced immunogenicity due to lower level of dsRNA**
- **GMP grade will be made available**

RECOMMENDED IVT CONDITION

Component	Volume
SYNTHGENE's New CAP (100mM)	2 µl
CTP / GTP/ ATP/ N1-Me-pUTP (100 mM each)	2 µl each
T7 RNA Polymerase (low dsRNA)	1 µl
5×Transcription Buffer (low dsRNA)	4 µl
Inorganic Pyrophosphatase (1 U/µL)	0.04 µl
Murine RNase inhibitor (40 U/µL)	1 µl
DNA Template	1 µg
RNase free H_2O	Up to 20 µl

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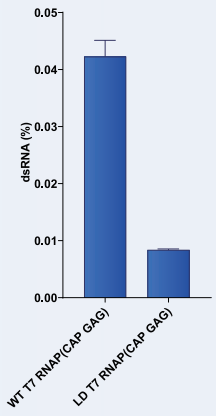
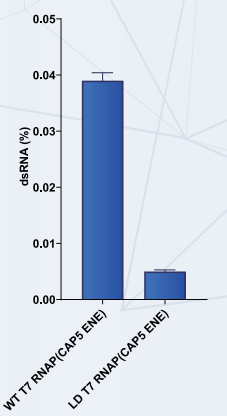
IVT PERFORMANCE

FLuc-eGFP mRNA was synthesized using both wild-type T7 RNA polymerase and T7 RNA Polymerase (low dsRNA) in a 20ul IVT reaction. CAP analogs were selected for CAP GAG and CAP5 ENE. Compared to WT T7 RNAP (CAP GAG), the LD T7 RNAP (CAP GAG) generated much lower dsRNA (0.0084% vs. 0.0423%), and LD T7 RNAP (CAP5 ENE) generated much lower dsRNA (0.005% vs. 0.039%) in comparison with the WT T7 RNAP (CAP5 ENE).



mRNA name	dsRNA
WT T7 RNAP(CAP GAG)	0.0423%
LD T7 RNAP(CAP GAG)	0.0084%

Fig 1. dsRNA Residue Detection of WT T7 RNAP(CAP GAG) and LD T7 RNAP(CAP GAG)



mRNA name	dsRNA
WT T7 RNAP(CAP5 ENE)	0.039%
LD T7 RNAP(CAP5 ENE)	0.005%

Fig 2. dsRNA Residue Detection of WT T7 RNAP(CAP5 ENE) and LD T7 RNAP(CAP5 ENE)

The following table shows yield, integrity and capping eficiency of CAP5 ENE. The results show that the yield, integrity and capping eficiency are up to the standard.

Table 1. T7 RNA polymerase (low dsRNA) evaluation data

mRNA name	260/280	Yield	Integrity	Capping rate
WT T7 RNAP(CAP5 ENE)	1.92	175x	92.5%	98.3%
LD T7 RNAP(CAP5 ENE)	1.92	179x	93.6%	98.5%

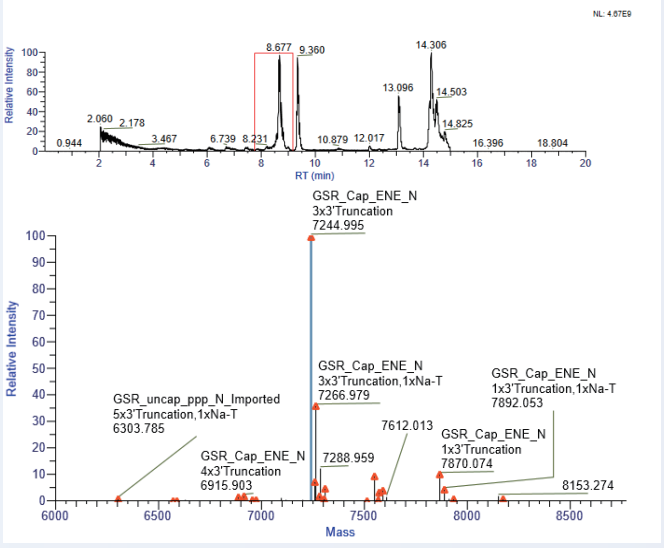


Fig 3. FLuc-eGFP mRNA by IVT using WT T7 RNAP(CAP5 ENE) capping rate is 98.3%

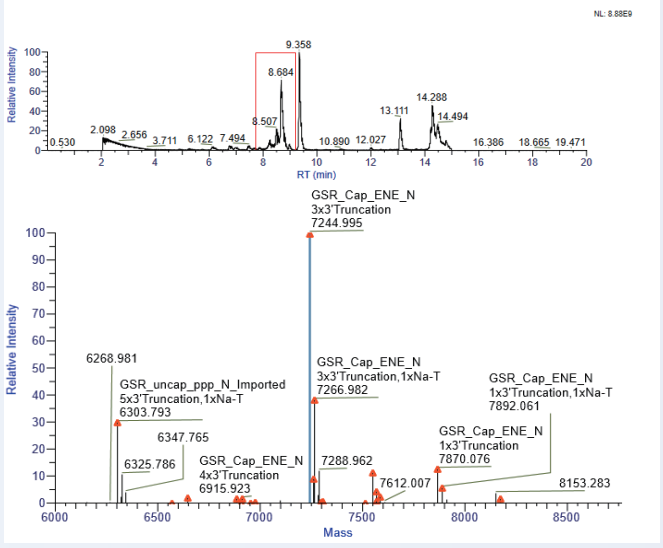


Fig 4. FLuc-eGFP mRNA by IVT using LD T7 RNAP(CAP5 ENE) capping rate is 98.5%

In summary, this T7 RNA Polymerase (low-dsRNA) generates lower dsRNA from IVT. With enhanced IVT performance, this product can help to make better mRNA for therapeutics.

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