



**Hongene Biotech**

NUCLEOSIDES & NUCLEOTIDES

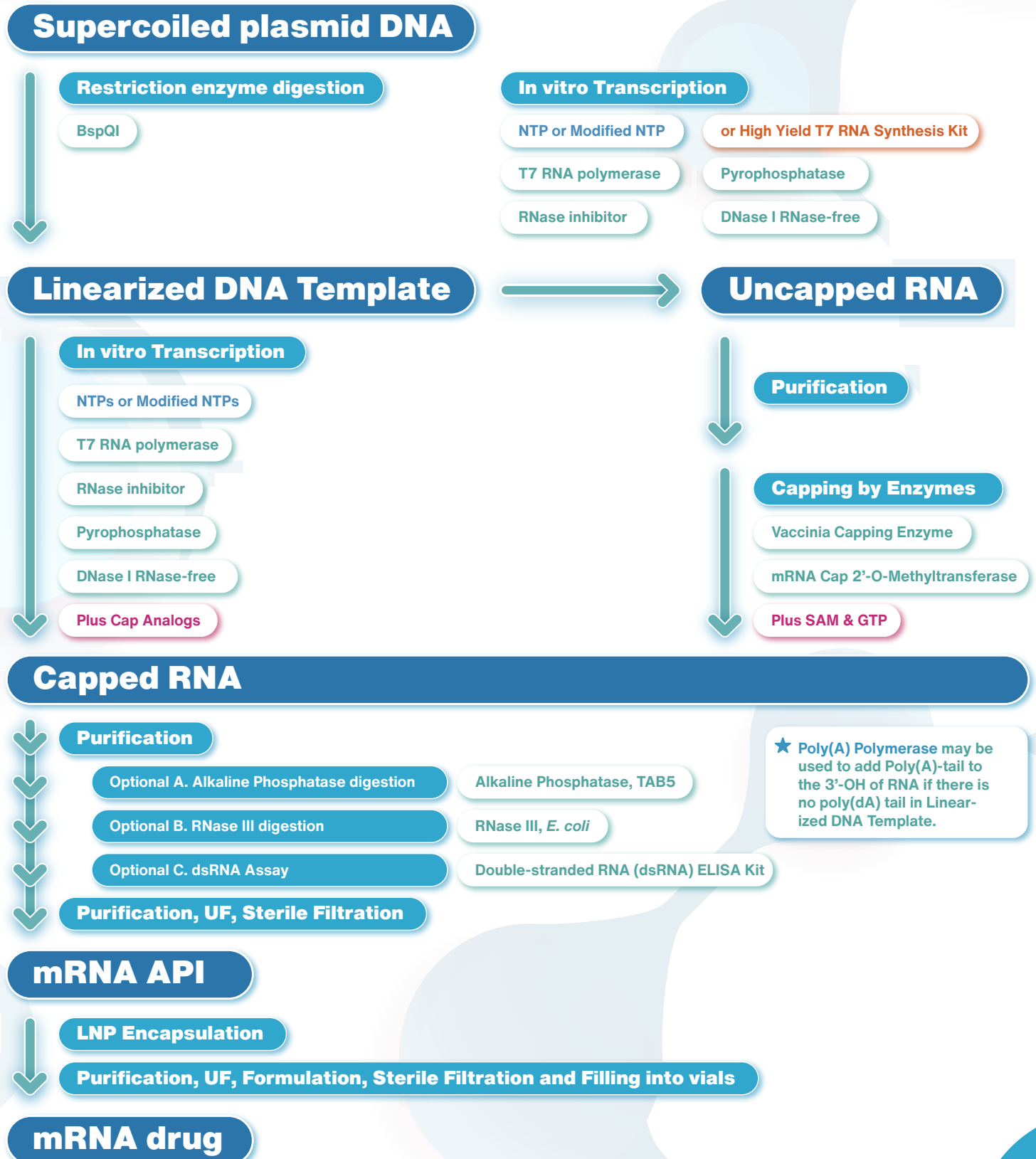
# **IN VITRO** **TRANSCRIPTION**

**PRODUCT**  
**CATALOG**  
**2022**  
**V 2.3**

# Content

<b>Schematic diagram of mRNA synthesis</b>	<b>01</b>
<b>Custom mRNA</b>	<b>02</b>
<b>BspQI (BSA-free)</b>	<b>03</b>
<b>High Yield T7 RNA Synthesis Kit</b>	<b>04</b>
<b>T7 RNA Polymerase</b>	<b>05</b>
<b>SP6 RNA Polymerase</b>	<b>06</b>
<b>Pyrophosphatase Inorganic (Yeast)</b>	<b>07</b>
<b>DNase I</b>	<b>08</b>
<b>RNase Inhibitor</b>	<b>08</b>
<b>Vaccinia Capping Enzyme</b>	<b>09</b>
<b>mRNA Cap 2'-O-Methyltransferase</b>	<b>10</b>
<b>Poly(A) polymerase</b>	<b>11</b>
<b>Other enzymes related to IVT</b>	<b>12</b>
<b>Nucleotides</b>	<b>13</b>
<b>Modified Nucleotides</b>	<b>13</b>
<b>Cap Analogs</b>	<b>13</b>

# Schematic diagram of mRNA synthesis



# Custom mRNA

## Good mRNA is far more than pure

Good mRNA should boost the translation after it is transfected or injected into cells, which requires a high integrity of the mRNA. In eukaryote, the cap1 structure at 5' end is vital because it promotes the translation, prevents the degradation and reduces the immunogenicity. The translation may be inhibited by the impurities and byproducts, such as the endotoxin, double stranded RNA, which are often be neglected.

Hongene mRNAs are produced under the control of GMP standard. Each mRNA is under a strict detection of the amount of dsRNA, capping efficiency, purity and integrity. So the mRNA is ready to be translated.

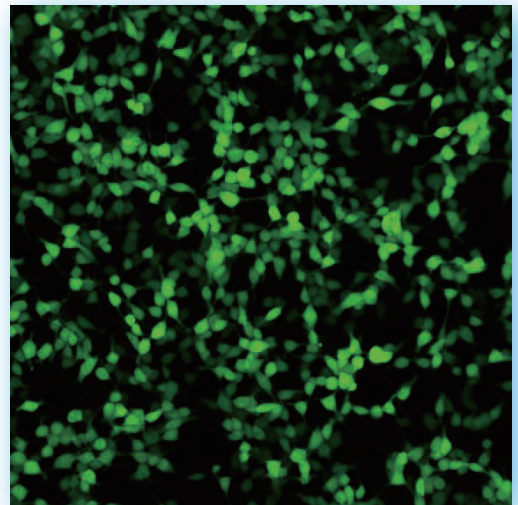
## Feature

- Integrity > 90%
- Cap1 added to the 5' end, with the capping efficiency up to 99.8%
- 100 nt poly(A) added to the 3' end
- A260/A280 = 1.8-2.0
- A260/A230 > 2.0
- Endotoxin < 1 EU/mg RNA
- dsRNA low to 0.002%
- Multiple choices for the modified nucleotides

Contact [info@hongene.com](mailto:info@hongene.com) to get the “**Product Requirement Document for mRNA Synthesis Service**”, and your own mRNAs.

## Alternative QC

**mRNA sequencing**  
**mRNA size**  
**Identity of Cap**  
**Poly(A) tail length**  
**Identity of modified bases**  
**Changes in base modification**  
**Identity of mRNA integrity**  
**Residual NTP, SAM, SAH**  
**Residual Enzymes**  
**Endotoxin**  
**Residual solvent**  
**Bioburden**  
**Residual host cell protein**  
**Residual host cell DNA**  
**Residual template DNA**  
**Concentration**  
**Capping efficiency**  
**More...**



### Expression of Hongene eGFP.

2.5 µg eGFP mRNA was transfected into  $1 \times 10^6$  HEK293T cell. 24 hours later, photos were taken under the fluorescent microscope.



# Plasmid linearization

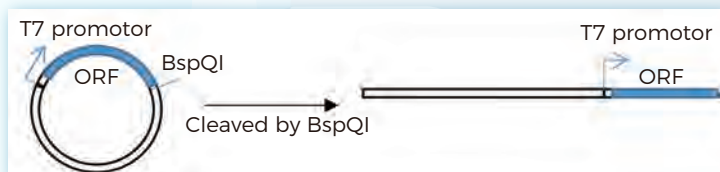
## — IIs restriction enzyme

### BspQI (BSA-free) - No BSA

**BspQI**, which is cloned from *Bacillus sphaericus*, is a IIs restriction enzyme. It recognizes and cleaves at 5'-GCTCTTCN<sub>1</sub>/N<sub>4</sub>-3'.



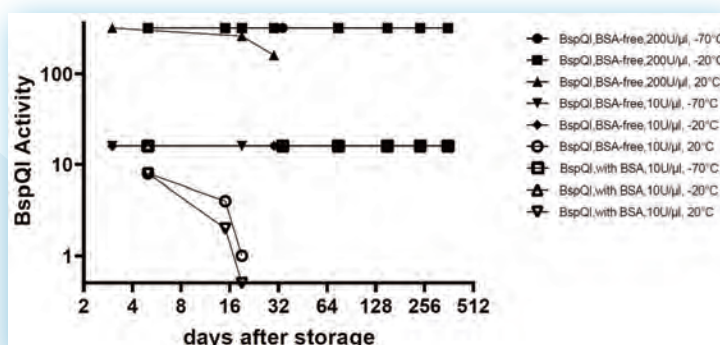
It is recommended to linearize the template plasmid DNA at the site you like by a restriction enzyme to make sure the transcripts can be terminated accurately. The IIs restriction enzymes, such as BspQI is the most popular one because it does not introduce additional sequence at the cleavage site therefore gives a clean polyA tail. Meanwhile, the 3' overhang is avoided on the linearized template, which can reduce the amount of unexpected transcripts and the double stranded RNA byproducts.



## Feature

- Animal-free, protein-free and endotoxin-free in reaction buffer.

## Standability



Standability of BspQI of different concentrations under different storage conditions. The enzyme activities were assayed at 3, 5, 15, 19, 30, 34, 75, 151, 241, 357 days after the first stor-

## Product information

### Unit definition

One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1X Cut Buffer following 1 hour incubation at 50°C in a total reaction volume of 50 μL.

### Concentration

10,000 units/mL

### Reaction Temperature

50°C

### Heat inactivation

80°C for 20 min

### Storage buffer

20 mM Tris-HCl  
1 mM DTT  
0.1 mM EDTA  
500 mM KCl  
0.1% Triton X-100  
50% Glycerol  
pH 7.0 @ 25°C

### Reaction buffer

10X Cut Buffer (BSA-Free)  
500 mM Tris-HCl  
1000 mM NaCl  
100 mM MgCl<sub>2</sub>  
pH 7.9 @ 25°C

## Order information

Cat No.	Description	Amount	Storage
ON-124	BspQI	10 KU	-70°C/-20°C
N/A	10X Cut Buffer (BSA-Free)	5 mL	-20°C

# RNA Synthesis—Kits

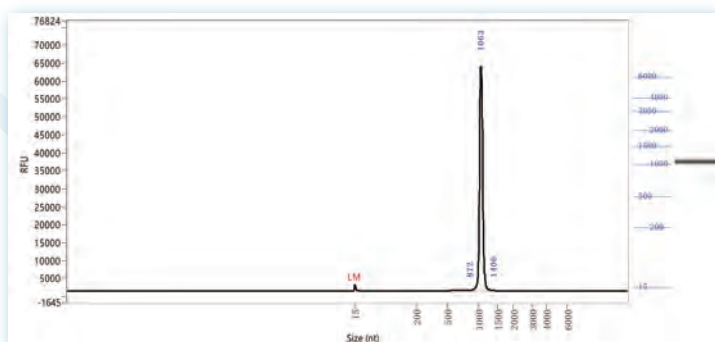
## High Yield T7 RNA Synthesis Kit

**High Yield T7 RNA Synthesis Kit** is designed to produce 25-fold more full-length RNA transcript per reaction than conventional in vitro transcription reactions. The yield is up to 210 µg RNA per reaction.

### Usage

- Synthesize transcripts in vitro
- Synthesize gRNA for gene editing
- Synthesize a mRNA with fluorescent or radio-labeled nucleotides
- Synthesize a mRNA with base-modified nucleotides
- Synthesize a capped mRNA with cap analog in 1 step

### Classic result



RNA synthesized with Hongene High Yield T7 RNA Synthesis Kit. The transcripts was cleanup by LiCl precipitation, and diluted by RNase-free water. 400 ng of the dilution was assayed with Agilent 5200 Fragment Analyzer.

### Order information

Cat No.	Description	Amount	Storage
ON-040	High Yield T7 RNA Synthesis Kit	50 rxns	-20°C

### Relevant Products

Cat No.	Description	Amount	Storage
R1331	ATP, 100 mM Sodium Solution	1 mL	-20°C
R2331	CTP, 100 mM Sodium Solution	1 mL	-20°C
R3331	GTP, 100 mM Sodium Solution	1 mL	-20°C
R5331	UTP, 100 mM Sodium Solution	1 mL	-20°C

## Procedure

### Template plasmid

↓ BspQI (BSA-free)

### Linearized Template

↓ NTPs

↓ Reaction buffer

↓ Enzyme Mix

↓ RNase-free water

### Crude RNA

↓ DNase I digest

### Crude RNA without template

↓ Cleanup

### RNA precipitation

↓ Dissolve

### Purified RNA

↓ QC

# RNA Synthesis—Enzyme

## T7 RNA Polymerase

**T7 RNA polymerase** is a monomeric bacteriophage encoded DNA directed RNA polymerase which catalyzes the formation of RNA in the 5' → 3' direction. In the process of initiation of transcription T7 recognizes a specific promoter sequence, the T7 promoter. T7 consists of 883 amino acids and has a molecular weight of 99 kDa.

**T7 promoter** **+1**  
**TAATACGACTCACTATAGGGAGA**

### Usage

- Synthesize the radio-labeled RNA probes
- Synthesize the RNA template for in vitro translation
- Research on RNA structure, process and catalytic mechanism

### Feature

- Produce high yields in natural NTPs as well as modified ones
- Capping efficiency up to 95% when transcribing with some cap analogs
- Provide customized concentration to meet special requirements

### Order information

Cat No.	Description	Amount	Storage
ON-004	T7 RNA polymerase	10 KU	-20°C
ON-062	10X IVT Reaction Buffer	5 mL	-20°C

### Relevant Products

Cat No.	Description	Amount	Storage
R1331	ATP, 100 mM Sodium Solution	1 mL	-20°C
R2331	CTP, 100 mM Sodium Solution	1 mL	-20°C
R3331	GTP, 100 mM Sodium Solution	1 mL	-20°C
R5331	UTP, 100 mM Sodium Solution	1 mL	-20°C

## Product information

### Unit definition

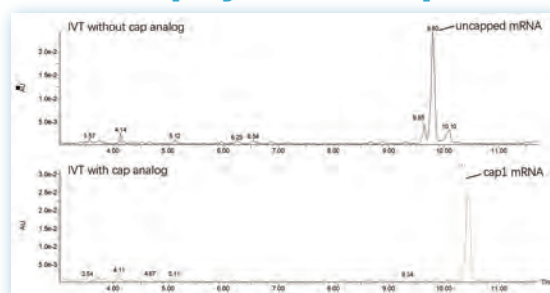
One unit is defined as the amount of enzyme required to catalyze the incorporation of 1 nmol of ATP into acid-insoluble product in 1 hour at 37°C in a total volume of 20 µL.

### Concentration

50,000 units/mL  
200,000 units/mL  
1,000,000 units/mL

Storage buffer	Reaction buffer
20 mM Tris-HCl	10X IVT
100 mM NaCl	Reaction Buffer
10 mM DTT	400 mM Tris-HCl
0.1% Triton X-100	100 mM DTT
1 mM EDTA	20 mM Spermidine
50%(v/v) Glycerol	60 mM MgCl <sub>2</sub>
	pH 7.9 @ 25°C

### Add 5'-cap by cotranscription



The transcriptional reaction without cap analog (upper) or with cap analog (down). After the reaction, the transcripts were annealed with 5' end DNA probes, digested by RNaseH. The purified 5' ends were assayed by HPLC. The capping efficiency was up to 95%.

# Alternative choice for in vitro transcription —SP6 RNA Polymerase

**SP6 RNA Polymerase** is cloned from *Salmonella typhimurium* LT2Z, and expressed recombinantly. SP6 RNA Polymerase has a molecular weight of 98.5 kDa. It specifically recognizes sp6 promoter (as below), which is an alternative choice for gene design.

**SP6 promoter** +1  
**ATTAGGTGACACTATAGAA**

**High Yield SP6 RNA Synthesis Kit** is a kit used for in vitro transcription with SP6 RNA Polymerase. It is designed to produce 8-fold more full-length RNA transcript per reaction up to 80 µg with a reducing enzyme and NTPs consumption. Reagents for transcription, template depletion, purification and electrophoresis are concluded in the kit.

## Usage

- Synthesize the radio-labeled RNA probes
- Synthesize the RNA template for in vitro translation
- Research on RNA structure, process and catalytic mechanism

## Feature

- Produce high yields in natural NTPs as well as modified ones.
- Capping efficiency up to 95% when transcribing with some cap analogs.

## Order information

Cat No.	Description	Amount	Storage
ON-338	High Yield SP6 RNA Synthesis Kit	50 rxns	-20°C
ON-297	SP6 RNA polymerase	2 KU	-20°C/-70°C
N/A	5X SP6 Reaction Buffer	0.5 mL	-20°C

## Product information

### Unit definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 1 nmol of ATP into acid-insoluble product in 1 hour at 37°C in a total volume of 20 µL.

### Concentration

20,000 units/mL

### Storage buffer

20 mM Tris-HCl  
100 mM NaCl  
20 mM DTT  
0.1% Triton X-100  
1 mM EDTA  
50%(v/v) Glycerol  
pH 7.9 @ 25°C



# RNA Synthesis—Enzyme

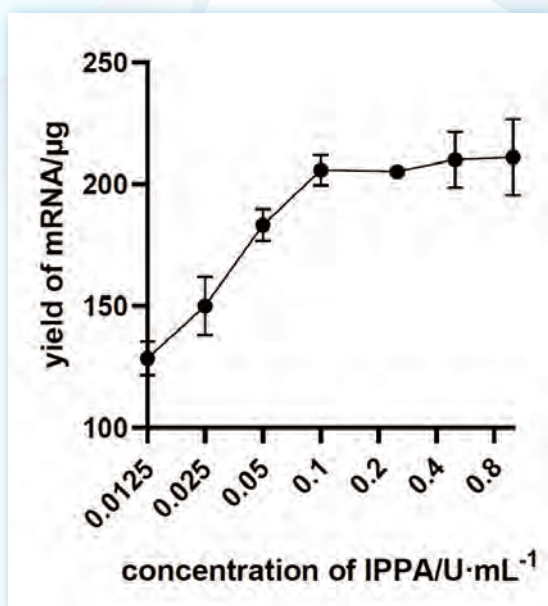
## Pyrophosphatase Inorganic (Yeast)

**Inorganic pyrophosphatase (Yeast)** is prepared from an *E. coli* strain containing *Saccharomyces cerevisiae ppa* gene. It is a homodimer consisting of two equal subunits of molecular weight 32-35 kDa. Inorganic pyrophosphatase catalyzes the following reaction:



The synthesis of RNA will generate pyrophosphate, which can bind  $\text{Mg}^{2+}$  to form sediment and result in a declined yield of RNA. However, under the action of pyrophosphatase, the pyrophosphate byproduct can be degraded into phosphate, which pulls the reaction far in the synthetic direction.

### IPPA influence the yield of transcription



Inorganic pyrophosphatase (Yeast) was added into a 20 µL IVT reaction at a final concentration of 0.0125-1 U/mL. The mRNA yield was enhanced.

## Product information

### Unit definition

One unit is the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions.

### Concentration

100 units/mL

### Storage buffer

20 mM Tris-HCl

1 mM DTT

0.1 mM EDTA

50% Glycerol

pH 8.0 @ 25°C

## Order information

Cat No.	Description	Amount	Storage
ON-025	Pyrophosphatase Inorganic (Yeast)	10 KU	-20°C

## Relevant Products

Cat No.	Description	Amount	Storage
ON-004	T7 RNA polymerase	10 KU	-20°C
ON-062	10X IVT Reaction Buffer	0.5 mL	-20°C
ON-039	Ribonuclease Inhibitor, Human Placenta	40 KU	-20°C

# Template Digestion —DNase I

**DNase I** is an endonuclease that digests single- and double-stranded DNA. It nonspecifically cleaves DNA to release di-, tri- and oligonucleotide products with 5'-phosphorylated and 3'-hydroxylated end.

## Usage

- **Digest the template DNA after transcriptional reaction**

## Order information

Cat No.	Description	Amount	Storage
ON-109	DNase I (RNase-free)	1000 U	-20°C
ON-077	10X DNase I Reaction Buffer	1 mL	-20°C

# Product information

### Unit definition

One unit is defined as the amount of Recombinant Ribonuclease Inhibitor required to inhibit the activity of 5 ng of ribonuclease A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2'3'-cyclic monophosphate by ribonuclease A.

### Concentration

40,000 units/mL

### Storage buffer

20 mM HEPES-NaOH  
50 mM NaCl  
8 mM DTT  
50%(v/v) glycerol  
pH 7.6 @ 25°C

# Product information

### Unit definition

One unit is defined as the amount of enzyme required to completely degrade 1 µg of λ DNA in 10 minutes at 37°C in a total volume of 50 µL.

### Concentration

1,000 units/mL

### Storage buffer

50 mM Tris-HCl  
10 mM CaCl<sub>2</sub>  
50% Glycerol  
pH 7.5 @ 25°C

### Reaction buffer

10X DNase I  
Reaction Buffer  
100 mM Tris-HCl  
25 mM MgCl<sub>2</sub>  
10 mM CaCl<sub>2</sub>  
pH 7.6 @ 25°C

# Prevent Degradation —RNase Inhibitor

**Ribonuclease Inhibitor, Human Placenta** is a recombinant human placental protein with specifically inhibits broad-spectrum RNase such as RNase A, RNase B and RNase C. But It is not effective against RNase I, RNase T1, S1 Nuclease, RNase H, Taq DNA polymerase, M-MLV Reverse Transcriptase and T7 RNA Polymerase. The 50kDa protein exerts its inhibitory effect by noncovalently binding to RNases at 1:1 ratio. The Ki value for binding of RNase Inhibitor, Human Placenta to RNase is approximately 10<sup>-14</sup> M.

## Usage

- **In vitro transcription/translation**
- **cDNA synthesis**
- **RNA labeling by enzymatic method**

## Order information

Cat No.	Description	Amount	Storage
ON-039	Ribonuclease Inhibitor, Human Placenta	40 KU	-20°C

# Capping

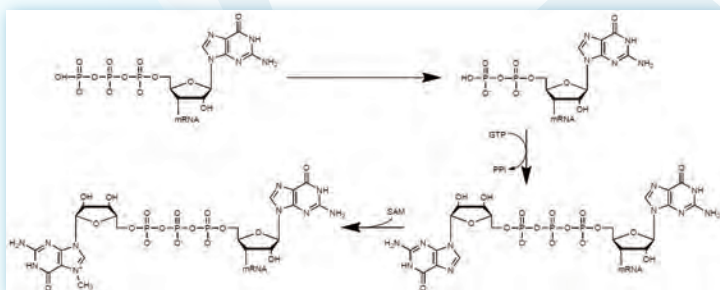
## —Vaccinia Capping Enzyme

**Vaccinia Capping Enzyme** is a recombinant protein from Vaccinia virus. Vaccinia Capping Enzyme can add 7-methylguanylate cap structure (Cap 0) to the 5' end of RNA.

In eukaryotes, these terminal cap structures are involved in stabilization, transport and translation of mRNAs.

RNA triphosphatase, RNA guanylyltransferase, and RNA (guanine-7)-methyltransferase activities are associated with the vaccinia virus RNA capping enzyme, a heterodimeric protein containing polypeptides of Mr 95,000 and Mr 31,000.

In vitro, transcripts can be capped in the presence of reaction buffer, GTP, and the methyl donor (SAM).



**Add 5'-cap by Vaccinia Capping Enzyme**

## Usage

- Add natural cap structure to 5' end of mRNA
- Label the 5' end of mRNA
- Synthesize Cap1 mRNA together with 2'-O-Methyltransferase

## Order information

Cat No.	Description	Amount	Storage
ON-028	Vaccinia Capping Enzyme	10 KU	-20°C
ON-073	10X Capping Buffer	2 mL	-20°C
ON-074	S-adenosylmethionine (SAM)	1 mL	-20°C
ON-075	10 mM GTP	1 mL	-20°C

## Product information

### Unit definition

One unit is defined as the amount of enzyme required to incorporate 10pmol of GTP into an 80 nt transcript in 1 hour at 37°C.

### Concentration

10,000 units/mL

### Storage buffer

20 mM Tris-HCl  
0.1 mM EDTA  
100 mM NaCl  
1 mM DTT  
0.1%(v/v) Triton X-100  
50% Glycerol  
pH 8.0 @ 25°C

### Reaction buffer

**10X Capping Buffer**  
500 mM Tris-HCl  
50 mM KCl  
10 mM MgCl<sub>2</sub>  
10 mM DTT  
pH 8.0 @ 25°C

### Brief protocol

1. Heat at 65°C for 5 minutes, Ice bath for 5 minutes.
2. Prepare a 20 µL reaction, 2'-O-Methyltransferase can be added simultaneously.
3. Incubation at 37°C for 30 minutes.

## Relevant Products

Cat No.	Description	Amount	Storage
ON-014	mRNA Cap 2'-O-Methyl Transferase	50 KU	-20°C

# Capping

## —mRNA Cap 2'-O-Methyl-transferase

**mRNA Cap-2'-O-Methyltransferase** is a recombinant protein from Vaccinia virus. The enzyme can add a methyl group at the 2'-O position of the first nucleotide adjacent to the Cap structure at the 5' end of the RNA. The enzyme utilizes SAM as a methyl donor to methylate capped RNA (Cap 0) resulting in a Cap 1 structure.

The Cap structure has been reported to enhance mRNA translation efficiency and hence may help improve expression in mRNA transfection and microinjection experiments.

mRNA cap-2'-O-Methyltransferase specifically requires RNA with an m7GpppN Cap as substrate.

## Usage

- **Transfer Cap0 mRNA into Cap1 mRNA**
- **Enhance translation efficiency of mRNA**

## Order information

Cat No.	Description	Amount	Storage
<b>ON-014</b>	mRNA Cap 2'-O-Methyltransferase	50 KU	-20°C
<b>ON-073</b>	10X Capping Buffer	2 mL	-20°C
<b>ON-074</b>	S-adenosylmethionine (SAM)	1 mL	-20°C

## Product information

### Unit definition

One unit is defined as the amount of enzyme required to methylate 10 pmols of 80 nts Capped RNA transcript in 1 hour at 37°C.

### Concentration

50,000 units/mL

### Storage buffer

20 mM Tris-HCl  
0.1 mM EDTA  
100 mM NaCl  
1 mM DTT  
0.1%(v/v) Triton X-100  
50% Glycerol  
pH 8.0 @ 25°C

### Reaction buffer

**10X Capping Buffer**  
500 mM Tris-HCl  
50 mM KCl  
10 mM MgCl<sub>2</sub>  
10 mM DTT  
pH 8.0 @ 25°C

# Tailing

## —Poly(A) polymerase

**Poly(A) Polymerase** uses ATP as a substrate for template-independent addition of adenosine monophosphate to the 3'-hydroxyl termini of RNA molecules.

## Usage

- Add polyA tail to transcripts
- Enhance the translation efficiency
- Label RNA with fluorescent or radio-labeled ATP

## Order information

Cat No.	Description	Amount	Storage
ON-126	Poly(A) Polymerase	5000 U	-20°C
ON-127	10X poly(A) Polymerase Reaction Buffer	2 mL	-20°C
R1331	ATP, 100mM Sodium Solution	1 mL	-20°C

## Relevant products

Cat No.	Description	Amount	Storage
ON-040	High Yield T7 RNA Synthesis Kit	50 rxns	-20°C
ON-039	Ribonuclease Inhibitor, Human Placenta	40 KU	-20°C

## Product information

### Unit definition

One unit is defined as the amount of enzyme that will incorporate 1 nmol of AMP into RNA in a 20 µL volume in 10 min at 37°C.

### Concentration

5,000 units/mL

### Storage buffer

20 mM Tris-HCl  
1 mM EDTA  
300 mM NaCl  
1 mM DTT  
0.1%(v/v) Triton X-100  
50% Glycerol  
pH 7.5 @ 25°C

### Reaction buffer

10X poly(A) Polymerase Reaction Buffer  
500 mM Tris-HCl  
2.5 M NaCl  
100 mM MgCl<sub>2</sub>  
pH 7.9 @ 25°C



# RNA Synthesis

## Nucleotides and Cap Analogs

**Hongene Biotech Corporation** has become a manufacturer with state-of-art facilities in the fields of nucleosides, nucleotides and phosphoramidites since 2001.

At Hongene our experts are also happy to offer custom synthesis products essential for your R&D, helping to bring your products to market more efficiently. Through continuous innovation, improvement of manufacturing technologies, the tightest quality control and devoted technical team and services, we aim to help our customers to achieve their goals efficiently and economically.

To customize cap analogs, please contact [info@hongene.com](mailto:info@hongene.com)

### NTPs

Cat No.	Description	Amount	Storage
<b>R1331</b>	ATP, 100 mM Sodium Solution	1 mL	-20°C
<b>R2331</b>	GTP, 100 mM Sodium Solution	1 mL	-20°C
<b>R3331</b>	CTP, 100 mM Sodium Solution	1 mL	-20°C
<b>R5331</b>	UTP, 100 mM Sodium Solution	1 mL	-20°C
<b>R5-027</b>	N1-Me- Pseudo UTP, 100 mM Sodium Solution	100 µL	-20°C
<b>R1-051</b>	ATP, 200 mM Tris Solution	100 µL	-20°C
<b>R2-057</b>	GTP, 200 mM Tris Solution	100 µL	-20°C
<b>R3-052</b>	CTP, 200 mM Tris Solution	100 µL	-20°C
<b>R5-065</b>	UTP, 200 mM Tris Solution	100 µL	-20°C
<b>R5-064</b>	N1-Me-Pseudo UTP, 200mM Tris Solution	100 µL	-20°C
<b>R3-029</b>	5-Me-CTP, 100mM Sodium Solution	1 mL	-20°C
<b>R5-104</b>	5-Me-UTP, 100mM Sodium Solution	1 mL	-20°C
<b>R5-022</b>	Pseudo UTP, 100mM Sodium Solution	1 mL	-20°C
<b>R5-046</b>	5-OMe-UTP, 100mM Sodium Solution	1 mL	-20°C
<b>R5-066</b>	5-OMe-UTP, 200mM Tris Solution	100 µL	-20°C

### Cap Analogs

Cat No.	Description	Amount	Storage
<b>ON-089</b>	GpppG	Inquiry	-20°C
<b>ON-137</b>	GpppA	Inquiry	-20°C
<b>ON-138</b>	M7-GpppA	Inquiry	-20°C
<b>ON-136</b>	M7-GpppG	Inquiry	-20°C
<b>ON-134</b>	GAG Trimer*	Inquiry	-20°C

\* The use of this product may require the buyer to obtain additional third party intellectual property rights for certain applications.

# Enzymes & Kits

## Kits

Cat No.	Description	Amount	Storage
ON-040	High Yield T7 RNA Synthesis Kit	50 rxns	-20°C
ON-257	Capped RNA Synthesis Kit*	N/A	-20°C
ON-269	Capped RNA Synthesis Kit (with tailing)*	N/A	-20°C

\* For sale outside USA only.

## Enzymes

Cat No.	Description	Amount	Storage
ON-124	BspQI (BSA-free)	10 KU	-70°C/-20°C
ON-211	T7 Enzyme mix	N/A	-20°C
ON-004	T7 RNA Polymerase, low concentration	10 KU	-20°C
ON-005	T7 RNA Polymerase, high concentration	N/A	-20°C
ON-039	RNase Inhibitor (recombinant)	40 KU	-20°C
ON-025	Pyrophosphatase, Inorganic (yeast)	10 KU	-20°C
ON-109	DNase I (recombinant, RNase-free)	1000 U	-20°C
ON-028	Vaccinia Capping Enzyme	10 KU	-20°C
ON-014	2'-O-Methyltransferase	50 KU	-20°C
ON-074	S-adenosylmethionine (SAM), 32mM	N/A	-20°C
ON-126	Poly(A) Polymerase	5000 U	-20°C
ON-024	RNase III, <i>E.coli</i>	1000 U	-70°C
ON-078	10X RNase III Reaction Buffer	1 mL	-20°C
ON-079	10X MnCl <sub>2</sub>	1 mL	-20°C
ON-080	10X EDTA	1 mL	-20°C
ON-081	T4 RNA Ligase 1	N/A	-20°C
ON-179	Alkaline Phosphatase, TAB5	1000 U	-20°C
ON-180	10X Phosphatase Reaction Buffer	0.8 mL	-20°C
ON-333	NLS-Cas9, 10 µM	1000 pmol	-70°C

**CDMO Services Available**



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***GMP Facility***  
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***Animal-FREE***