Ver. HB240509

CleaScript™ T7 RNA Polymerase (low dsRNA, 250 U/μL)

Product description

This is an engineered T7 RNA polymerase variant (low dsRNA) derived from the wild-type T7 RNA polymerase and produced in Escherichia coli. It significantly reduces the production of double-stranded RNA (dsRNA) while efficiently incorporating cap analogs, and exhibiting highly efficient in vitro transcription (IVT) comparable to the wild-type T7 RNA polymerase. It catalyzes the 5'→3' synthesis of RNA on double-stranded DNA from its T7 promoter sequence (5'-TAATACGACTCACTATAG*-3') and uses NTPs as substrates.

Note: G* is the first base of the RNA transcript.

Components

Components No.	Name	10628ES10 (10 KU)	10628ES60 (100 KU)	10628ES72 (2,50KU)	10628ES86 (2,500 KU)
10628	CleaScript™ T7 RNA Polymerase (low dsRNA, 250 U/μL)	40 μL	400 μL	1 mL	10 mL

Specifications

Source	Recombinant *E. coli* with T7 RNA Polymerase gene
Optimum Temperature	37°C
Storage Buffer	50 mM Tris-HCl, 1 mM EDTA, 10 mM DTT, 100 mM NaCl, 0.1% Triton X-100，50% (v/v) glycerin，pH7.9 at 25°C
Unit Definition	The amount of enzyme required to incorporate 1 nmol of [^{3}H] GMP into the acid-insoluble precipitate within 1 hour at 37°C and pH 8.0 is defined as 1 unit.

Shipping and Storage

The products are shipped with dry ice and can be stored at -15°C ~ -25°C for one year.

Instructions

1. *In vitro* transcription without cap-analog

1.1 Combine the following reaction components:

Component	Volume（μL）	Final concentration
RNase free H_2O	Up to 20	-
10× Transcription Buffer	2	1×
CTP / GTP/ ATP/ UTP (100 mM each)	2 each	10 mM each
CleaScript™ T7 RNA Polymerase (low dsRNA, 250 U/μL)	1	-

Pyrophosphatase, Inorganic (1 U/μL)	0.04	
RNase inhibitor (40 U/μL)	0.5	-
DNA template	2 (100 ng-1 μg)	-

Note: 1.1.1 The DNA template should be added last, because the 10×Transcription Buffer contains high concentration of spermidine, which may cause precipitation of DNA templates.

1.1.2 It is recommended to keep the buffer and water at room temperature before use. The reaction mix should be prepared at room temperature because spermidine will cause precipitation of high-concentration DNA templates at low temperature.

1.1.3 If the transcript is less than 100 nt, the templates should be increased to 2μg.

1.1.4 If you need RNase inhibitor (40 U/μL), please purchase our company's product cat#10621.

1.1.5 If you need Pyrophosphatase, Inorganic (1 U/μL), please purchase our company's product cat#10611.

1.1.6 In order to ensure effective transcription of a specific region, it is recommended to cut the DNA template downstream region into blunt ends or 5'protruding ends.

1.2 Incubate at 37°C for 2-4 h (if the transcript is less than 100 nt, increase the incubating time to 4-8 h).

1.3 After the reaction, add 2U DNase I (Cat#10611) and incubate at 37°C for 15-30 min to degrade the DNA template.

1.4 Purification of transcripts: RNA Cleaner magnetic beads(Cat#12602) can be used for purification of transcription products via removing proteins, salt ions and other impurities. Phenol/chloroform purification method can also be used (the specific steps can be obtained by contacting Yeasen).

2. *In vitro* transcription with cap1-analog

2.1 Combine the following reaction components:

Component	Volume（μL）	Final concentration
RNase free H_2O	Up to 20	-
10× Transcription Buffer	2	1×
CTP / GTP/ ATP/ UTP (100 mM each)	2 each	10 mM each
Cap1-analog (100 mM)	2	10 mM
CleaScript™ T7 RNA Polymerase (low dsRNA, 250 U/μL)	1	-
Pyrophosphatase, Inorganic (1 U/μL)	0.04	
RNase inhibitor (40 U/μL)	0.5	-
DNA template	2 (100 ng-1 μg)	-

Note: If you need Cap1-analog, please contact our company.

2.2 Incubate at 37°C for 2-4 h.

2.3 After the reaction, add 2U DNase I (Cat#10611) and incubate at 37°C for 15-30 min to degrade the DNA template.

2.4 Purification of transcripts: RNA Cleaner magnetic beads(Cat#12602) can be used for purification of transcription products via removing proteins, salt ions and other impurities. Phenol/chloroform purification method can also be used (the specific steps can be obtained by contacting Yeasen).

Notes

1. Types of DNA templates: It is recommended to use linearized plasmids or PCR products containing T7 promoters as templates.

2. The purity of the DNA template will significantly affect the yield of *in vitro* transcription. Residual RNase A during

the plasmid DNA extraction process will significantly affect the quality of transcribed RNA. Plasmid DNA template are recommended to be extracted by phenol-chloroform; PCR products are recommended to be purified by gel.

3. For your safety and health, please wear personal protective equipment (PPE), such as coats and gloves.