T7 RNA Polymerase

REF: EG20124-S/M

Storage Condition

-20℃

Components

Component	EG20124S	EG20124M
T7 RNA Polymerase (50 U/μl)	100 μl	500 μl
10×T7 RNA Polymerase Buffer	1.25 ml	1.25 ml

Description

T7 RNA Polymerase is derived from recombinant expression in *E.coli*. It is a DNA-dependent RNA polymerase that exhibits high specificity for the promoter sequence of bacteriophage T7. T7 RNA Polymerase utilizes double-stranded DNA templates containing the T7 promoter sequence and NTPs as substrates to synthesize single-stranded RNA complementary to the downstream of the promoter.

Definition of Activity Unit

One unit is defined as the amount of enzyme that incorporate 1 nmol of ATP into acid-insoluble material in 1 hour at 37℃ .

Applications

1. Synthesis of single-stranded RNA, including mRNA, siRNA, gRNA, and other types of RNA precursors.
2. Synthesis of labeled or unlabeled highly specific RNA probes.
3. Synthesis of capped mRNA using cap analogs.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 1 μg of supercoiled plasmid and 50 U of T7 RNA Polymerase incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-Specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 50 U of T7 RNA Polymerase incubated for 16 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of total RNA and 50 U of T7 RNA Polymerase incubated for 1 hours at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Protocol

1. Prepare the following reaction mixture on ice:

Reagent	Amount	Final Concentration
10×T7 RNA Polymerase Buffer	2 μl	1×
CTP/ GTP/ ATP/ UTP (100 mM each)	0.1~0.4 μl each	0.5~2 mM each
RNase Inhibitor (40 U/μl)	0.5~1 μl	1~2 U/μl
Template DNA	0.1~1 μg	-
T7 RNA Polymerase (50 U/μl)	1~2 μl	-
Nuclease-Free Water	up to 20 μl	-

Note: It is recommended to add Nuclease-Free Water first, followed by CTP/GTP/ ATP/UTP.

2. Mix gently and spin down, then incubate at 37℃ for 1 h. If the length of transcript is less than 300 nucleotides, the reaction time can be extended to 2~16 hours.
3. After the in vitro transcription (IVT) reaction, add 1 μl of dsDNase (REF: EG20206) to the product and incubate at 37℃ for 15 minutes to remove the DNA template.

Notice

1. The purity of the template DNA is crucial for IVT reactions. Residual RNase A introduced during plasmid DNA extraction can significantly affect the quality of transcribed RNA. It is recommended to use high-purity RNase-free template with an A_{260}/A_{280} ratio of 1.8~2.0.
2. The template DNA can be obtained from linearized circular plasmids or PCR. The upstream region of the template DNA should contain a T7 promoter sequence, while the downstream region should have a blunt end or a 5'-overhang.
3. For your safety and health, please wear a lab coat, disposable gloves, and a mask while conducting the experiment.

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