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T7 RNA Polymerase

Product Number: TR01

Shipping and Storage

-20℃.

Components

Components	TR01
T7 RNA Polymerase（50U/μl）	100μl
5×Transcription Buffer	1ml

Description

T7 RNA polymerase is a DNA dependent 5'→ 3'rna polymerase that highly specifically recognizes the T7 promoter sequence. T7 RNA polymerase can catalyze the incorporation of NTP downstream of T7 promoter of single stranded or double stranded DNA to synthesize RNA complementary to template DNA downstream of T7 promoter.

Source

It is expressed by Escherichia coli and the expressed gene is bacteriophage T7 RNA polymerase gene.

Concentration

50U/μl

Purity

No DNA endonuclease and exonuclease, no RNase.

Feature

T7 RNA polymerase can recognize modified NTPs, such as biotin labeled, digoxigenin labeled, fluorescein labeled NTPs, and can be used for the synthesis of various labeled RNAs. At the same time, it has high specificity for T7 promoter.

Application

For RNA synthesis, the synthesized RNA can be used or used as: hybridization probe, genomic DNA sequence analysis, RNase protection assay, antisense RNA synthesis, as RNA template for in vitro translation, substrate for RNA splicing research, RNA secondary structure and RNA protein interaction, nucleic acid amplification analysis, siRNA, miRNA and other small RNAs.

Unit definition

The amount of enzyme required to catalyze the incorporation of 1 nmol of AMP into polynucleotides within 60 min at 37 ° C was defined as 1 active unit.

Inactivation or inhibition

Heating at 70℃ for 10 min can inactivate T7 RNA polymerase. Addition of an appropriate amount of EDTA can also inactivate T7 RNA polymerase. Chelating agents, sodium, potassium or ammonium salts with concentrations greater than 150mm can significantly inhibit the activity of T7 RNA polymerase.

Protocol

1. RNA synthesis:

1.1. The DNA template was tangentially digested by restriction endonucleases.

1.2. DNA was extracted with phenol / chloroform, precipitated with ethanol, and dissolved in an appropriate amount of sterile deionized water. This step can also be directly purified using an appropriate DNA purification kit, thus eliminating the steps of phenol chloroform extraction and ethanol precipitation.

1.3. Refer to the following table to set up the reaction system:

Reagent	Volume
5×Transcription Buffer	10μl
NTP Mixture (10mM each)	10μl
Linear DNA template	1μg
Ribonuclease Inhibitor	50U
T7 RNA Polymerase	30U
Supplement DEPC treated deionized water	Up to 50μl

1.4. After setting up the reaction system according to the table above, mix it gently (you can use a pipette to blow it or use vortex to mix it gently at the lowest speed), and then centrifuge to precipitate the liquid.

1.5. Incubate for 1~2 hours at 37ºC.

1.6. Add 2μl of 0.5m EDTA (pH 8.0) to the reaction system and mix well or cool at -20ºC to terminate the reaction.

1.7. Electrophoretic analysis of transcripts, or other appropriate methods to identify the efficiency of transcription

Note: 1) transcription needs to be carried out without RNase.

2) The reaction system needs to be configured at room temperature, and DNA can precipitate in the presence of spermidine at 4ºC.

3) According to the above reaction conditions, more than 10 μ g of RNA can be synthesized per 1 μ g of template DNA.

4) If the template DNA is not fully linearized, it will lead to the transcription of RNA longer than the expected length, and the proportion of transcripts with the expected length will decrease.

5) The above reaction system can be scaled up or down according to the actual situation

2. Synthesis of radiolabeled RNA:

2.1. The DNA template was tangentially digested by restriction endonucleases.

2.2. DNA was extracted with phenol / chloroform, precipitated with ethanol, and dissolved in an appropriate amount of sterile deionized water. This step can also be directly purified using an appropriate DNA purification kit, thus eliminating the steps of phenol chloroform extraction and ethanol precipitation.

2.3. Refer to the following table to set up the reaction system:

Reagent	Volume
5×Transcription Buffer	4μl
3 NTP Mixture (10mM each , without CTP)	1μl
100μM CTP	2.4μl
[α- 32P]-CTP, ~30TBq/mmol(800Ci/mmol)	1.85MBq(50μCi)
Linear DNA template	0.2~1μg
Ribonuclease Inhibitor	20U
T7 RNA Polymerase	20U
Supplement DEPC treated deionized water	Up to 20μl

2.4. Incubate for 1~2 hours at 37ºC.

2.5. The reaction was terminated by cooling at -20ºC.

2.6. The labeling efficiency of RNA was analyzed and detected.

Note: 1) the RNA activity synthesized according to the above method is generally 3-5×10^8 dpm/ μG.

2) Other NTPs labeled with [32p], [35s] or [3h] can also be used in the above labeling reaction. When using other radiolabeled NTPs, other NTPs need to be adjusted accordingly. The recommended dosage of each component of

the 20μl reaction system is 1.85MBq (50μCi) 5'-[α- 32P]-CTP, ~30TBq/mmol(800Ci/mmol)； 11.1MBq (300μCi) 5'-[α- 35S]-UTP, >37TBq/mmol (>1000Ci/mmol) ； 0.925MBq (25μCi) 5,6-[3 H]-UTP, 1.1-2.2TBq/mmol (30-60Ci/mmol).

3) The efficiency of full-length transcript synthesis also decreased when the concentration of radiolabeled NTP was lower than 12μM.

3. Other uses can refer to the above uses or relevant literature.

Note

1. The enzyme should be stored in an ice box or on an ice bath when used, and should be stored at -20 ℃ immediately after use.
2. This product is limited to the scientific research of professionals, and shall not be used for clinical diagnosis or treatment, food or drugs, or stored in ordinary houses.
3. For your safety and health, please wear lab clothes and disposable gloves