T7 RNA Polymerase (thermostable)

Cat.No.:HYJ213

Product Description

T7 RNA Polymerase (thermostable) is suitable for in vitro transcription at elevated temperatures. Using a linear double-stranded DNA template containing the T7 promoter sequence and NTPs as substrates, it transcribes the DNA sequence downstream of the promoter to produce RNA. This enzyme can also incorporate modified nucleotides to produce biotin-, dye-, or radioactively labeled RNA, or use capping structures or analogs to produce capped RNA. The high-temperature reaction helps to reduce the formation of dsRNA byproducts and minimizes the immunogenicity of the resulting RNA product.

Components

Components	5KU	50KU	500KU	5000KU
T7 RNA Polymerase (thermostable) 50 U/μL	0.1 mL	1 mL	10 mL	100 mL
10×Hi-Yield IVT Buffer A	2 ×0.1 mL	2 ×1 mL	2 × 10 mL	2 × 100 mL

Storage

Transport below 0°C; store at -25 to -15°C. Valid for 24 months.

Storage buffer

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

Reaction system and conditions

Components	Volume
10×Hi-Yield IVT Buffer A	2 uL
ATP/GTP/CTP/UTP (100 mM each)	2 μL each (10 mM each Final)*
RNase inhibitors	1 μL (40 U)
Pyrophosphatase Inorganic	0.5 μL (0.05U)
T7 RNA Polymerase (low dsRNA) 50 U/μL	1 μL
Linearized template DNA	1 μg
Nuclease-free water	Up to 20 uL

Add the reaction components in the order listed above.

Reaction time: 37 °C for 2 hours, or 50 °C for 1 hour if high temperature reaction is required.

Termination reaction： Add 2 μL of 0.2 M EDTA (pH = 8.0@25°C)

DNA template removal: DNA template can be removed with 10U of DNase I and incubation for 30 min at 37 °C.

Notes

1. The transcription reaction should be performed in an environment free of RNase contamination. Wearing gloves is advisable. The tips, tubes and water should be nuclease free.
2. The transcription efficiency varies across different sequences, and gradient adjustments can be made to the reaction system.
3. The RNA synthesis reaction mixture should be prepared at room temperature, since DNA may precipitate in the presence of 10×Hi-Yield IVT Buffer A at 4°C.
4. The yield of proper length transcripts decreases if the template DNA is incompletely linearized.
5. The reaction mixture can be scaled up or down.
6. Transcribed fragments are less than or equal to 500bp, and it is recommended to extend the transcription time to 4-8 h.
7. Precipitates are easy to be found in 10×Hi-Yield IVT Buffer A after freezing and thawing, please check 10×Hi-Yield IVT Buffer A all dissolved before experiment. If precipitates are hard to be dissolved, redissolve 10×Hi-Yield IVT Buffer A at 37°C.