Hi-yield T7 in vitro transcription reagent (Thermostable)

Cat.No.:HYJ244

Product Description

T7 Thermostable RNA Polymerase can perform in vitro transcription at elevated temperatures (50°C). Using linear double-stranded DNA containing the T7 promoter sequence as a template and NTPs as substrates, it transcribes the DNA sequence downstream of the promoter to produce RNA products.

T7 Thermostable RNA Polymerase can also use modified nucleosides as substrates to produce RNA labeled with biotin, dyes, or radioactive substances, and capped RNA using cap- or cap-like structures as substrates. High temperatures help minimize the formation of dsRNA byproducts and reduce the immunogenicity of the transcribed RNA.

This kit contains the commonly used modified nucleoside N1-Me-pUTP for your transcription needs.This kit can produce 150–200 μg of RNA product per reaction and is suitable for scale-up to produce milligram quantities of RNA. The transcribed RNA can be used for RNA structure and function studies, RNase protection, probe hybridization, anti-sense RNA, and RNAi applications. It can also be capped using the vaccinia virus capping system (HYJ231) and 2'-O-methyltransferase (HYJ232), and tailed using Poly(A) Polymerase (HYJ233) to generate mRNA for downstream applications such as in vitro translation and transfection.

Components

Components	Concentration	50T
ATP	100 mM	100 μL
CTP	100 mM	100 μL
GTP	100 mM	100 μL
UTP	100 mM	100 μL
N1-Me-pUTP	100 mM	100 μL
10×Hi-Yield IVT Buffer A	/	100 μL
Enzyme Mix (Thermostable)	/	100 μL
Control template 1	0.5 μg/μL	10 μL
LiCl	5 M	1.4 mL
DNase I	5 U/μL	100 μL

Storage

Store at -25 to -15°C. Valid for 1 years.

Instructions

1. Template preparation

Linearized plasmids, PCR products, or synthetic DNA fragments containing a T7 promoter can be used as templates for in vitro transcription using the High-Yield T7 RNA Synthesis Kit. Templates can be dissolved in TE buffer or RNase-free ddH_2O.

1) Plasmid Template

A linearized plasmid with a T7 promoter can be used as a transcription template. The linearization and purity of the plasmid will affect the yield and integrity of the transcribed RNA product. Circular plasmids lack effective termination, resulting in RNA products of varying lengths. To obtain RNA of a specific length, the plasmid must be completely linearized. The linearized plasmid should ensure that both strands have blunt ends or 5' overhangs. After linearization, it is recommended to purify the plasmid before using it as a template for in vitro transcription to minimize the effects of RNase, protein, and salt residues.

2) PCR Product Template

A PCR product with a T7 promoter can be used as a template for in vitro transcription. During PCR amplification, the T7 promoter is added to the 5' end of the upstream primer on the noncoding strand. Purifying the PCR product as a template will yield higher RNA yields.

3) Synthetic DNA Template

Synthetic DNA fragments with a T7 promoter can also be used as templates for in vitro transcription.

2. RNA synthesis methods

Wear gloves during experimental procedures and use nuclease-free reaction tubes to avoid RNase contamination. For small-volume reactions, it is recommended to perform them in nuclease-free PCR tubes or 8-tube strips.

Conventional RNA Synthesis

1) Thaw all components on ice, mix thoroughly, and centrifuge briefly to collect the contents at the bottom of the tube. Store on ice until needed.
2) If performing multiple reactions simultaneously, mix equal volumes of 10× Hi-Yield IVT Buffer A and NTPs to create a Mix. Add 10 μL of Mix to each reaction.

3) At room temperature, add the samples in the order listed in the table below:

Components	Volume
10 × Hi-Yield IVT Buffer A	2 uL
ATP/CTP/GTP/UTP * (100 mM each)	2 μL each (10mM each Final)
Template DNA	X μL (1 μg)
Enzyme Mix	2 μL
Nuclease-free water	Up to 20 μL

*To reduce the immunogenicity of the product, an equal volume of UTP can be replaced with N1-Me-pUTP included in this kit.

4) Mix thoroughly, centrifuge briefly, and incubate at 37°C for 2 hours.

5) DNA Digestion: To remove the DNA template, add 10 U of DNase I (HYJ234) per 20 μL reaction, mix thoroughly, centrifuge briefly, and incubate at 37°C for 30 minutes.

Capped RNA Synthesis

Follow the standard RNA synthesis procedure, except for the addition system. Add samples at room temperature according to the order listed in the table below.

Components	Volume
10×Hi-Yield IVT Buffer A	2 uL
ATP/CTP/GTP/UTP (100 mM each)	2 μL each (10mM each Final)
Cap analog (100 mM)*	1.6 μL
Template DNA	X μL (1 μg)
Enzyme Mix	2 μL
Nuclease-free water	Up to 20 μL

*This kit does not contain cap analogs. If required, you can purchase Hyasen Cap Analogs cap1 (3'OH AG) (HYJ322) and cap1 (3'OMe AG) (HYJ323).

3. Product purification

Phenol/Chloroform Purification

Phenol/chloroform purification removes proteins and free nucleotides.

1) Add 160 μL of RNase-free ddH_2O to dilute the reaction product to 180 μL. Add 20 μL of 3M sodium acetate (pH 5.2) and mix thoroughly by pipetting.

2) Add an equal volume of phenol/chloroform mixture (1:1) for extraction. Centrifuge at 10,000 rpm for 5 minutes at room temperature. Transfer the supernatant to a new EP tube.
3) Add an equal volume of chloroform to the supernatant and extract twice. Collect the supernatant.
4) Add 2 volumes of anhydrous ethanol, mix thoroughly, incubate at -20°C for at least 30 minutes, centrifuge at 4°C for 15 minutes, and discard the supernatant.
5) Wash the RNA precipitate with 150-200 μL of 70% ice-cold ethanol. Centrifuge at 4°C for 15 minutes and discard the supernatant.
6) Uncap and dry for 2 minutes. Add 100-200 μL of RNase-free ddH_2O or other buffer to dissolve the RNA pellet.

Lithium chloride purification

Lithium chloride purification can remove proteins and most free nucleotides.

1) Add an equal volume of lithium chloride precipitation solution (5M) to the reaction product;
2) Mix well, precipitate at -20°C for 30 minutes, centrifuge at 12,000 rpm at 4°C for 15 minutes, and discard the supernatant;
3) Wash the RNA pellet with 200 μL of 70% ice-cold ethanol, centrifuge at 12,000 rpm at 4°C for 15 minutes, discard the supernatant, and repeat 2-3 times;
4) Uncap and air dry for 5-10 minutes. Once completely dry, add 100-200 μL of RNase-free ddH_2O or other buffer to dissolve the RNA pellet.

Column purification

Column purification can remove proteins and free nucleotides.

Magnetic bead purification

Magnetic bead purification can remove proteins and free nucleotides.

4. RNA Quantification

UV absorption method: Free nucleotides can affect quantification accuracy.This method requires RNA purification before use. The yield of in vitro transcribed RNA is then determined by measuring the A_{260} reading of the product.For single-stranded RNA, 1 A_{260}≈40 μg/mL.

Dye method: RNA quantification using RiboGreen dye is performed. Free nucleotides do not affect quantification, allowing accurate quantification of RNA in both purified and unpurified reaction products.

Notes

1. The optimal final concentration of NTPs in the reaction system is 10 mM. The actual amount used should be adjusted based on the original NTP concentration.

2. The transcription reaction should be performed in a nuclease-free environment. It is recommended to wear gloves and use nuclease-free water, pipette tips, and reaction tubes during the reaction.
3. The reaction system should be prepared at room temperature to avoid precipitation of DNA with spermidine at 4°C.
4. If insoluble material such as crystals or precipitates is observed in the buffer after reconstitution, vortex the buffer until the insoluble material is completely dissolved before use.
5. Incomplete linearization of the template DNA may reduce the yield and integrity of the transcript.
6. For RNA less than 300 nt, 2 μg of template can be used, and the transcription time can be increased to 4-8 hours.
7. The reaction volume can be scaled up or down based on actual needs.
8. In conventional systems, DNA templates are often designed with a GGG start sequence because T7 RNA polymerase has a higher affinity for GTP. This product's high-yield T7 in vitro transcription reagent comes with Control Template 1, which is a DNA template with a GGG start sequence.
9. For co-transcription systems, the recommended DNA template design is to use AGG as the starting sequence. The co-transcription capping T7 in vitro transcription reagent includes a control template 2, which is a DNA template with AGG as the starting sequence.