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T7 High Yield RNA Transcription kit

Product Number: E131

Storage

-20℃

Component

Component	Volume
Enzyme Mix	50μl
10×Transcription Buffer	200μl
ATP (100 mM)	80μl
UTP (100 mM)	80μl
GTP (100 mM)	80μl
CTP (100 mM)	80μl
Control template (100ng/μl)	10μl
Lithium Chloride Precipitation Solution	0.75ml×2
DNase I, RNase-free(1U/μl)	50μl
RNase Free Water	1ml

Description

As biological macromolecules, mRNA can be synthesized on a large scale through in vitro transcription (IVT,in vitro transcription). The T7 promoter is currently one of the most efficient promoters for transcription, so using T7 RNA polymerase (TR01,T7 RNA Polymerase) for in vitro transcription can easily and quickly obtain a large number of RNA molecules.This kit has been optimized through a series of transcription reaction systems, and RNA complementary to a single strand in DNA is synthesized from downstream of the template DNA T7 promoter using T7 RNA Polymerase. The operation is simple and fast.

This reagent kit can transcribe and produce 150-200μg RNA in one reaction. The synthesized RNA can be used for downstream applications such as RNA structure and function research, RNA enzyme protection, probe hybridization, RNAi, microinjection, and in vitro translation.

Application

1. Synthesis of single stranded RNA;
2. Synthesis of highly specific RNA probes;
3. Synthesis of siRNA precursors;
4. Produce precursors for RNA splicing reactions.

Note

1. Template efficiency and incubation time:
    1.1. This kit can produce 150-200μg RNA with a template input of 1μg. However, the yield of different templates may vary depending on the sequence, structure, length, purity of the template, as well as the sequence and length of specific RNA polymerase promoters.
2. Optimized response:
    2.1. The recommended reaction conditions can be applied to in vitro transcription of most templates, but for some templates, the yield can be increased by extending the reaction time (4 hours overnight reaction) and increasing the amount of templates used.
3. Template content:

3.1. The following table summarizes our experience in regulating the number of templates. The results may vary depending on the template used, and extending the reaction time to 4-6 hours can increase RNA production.

Template quantity	RNA production
1000ng(1μg)	130-160μg
500ng (0.5μg)	110-130μg
100ng (0.1μg)	30-50μg
50ng(0.05μg)	15-25μg
10ng(0.01μg)	10-20μg
1ng(0.001μg)	3-8μg

4. Maintain RNase free environment:
   4.1. Use RNase free tubes and pipettes;
   4.2. When handling kit components or samples containing RNA, gloves should be worn and replaced frequently, especially after coming into contact with potential sources of RNase contamination, such as door handles, pens, pencils, and human skin.
   4.3. When not in use, all reagents should be sealed properly. During the incubation process, seal all tubes containing RNA.
5. Due to 10×The Transcription Buffer contains spermidine components that can precipitate with the template DNA at low temperatures. When preparing the reaction solution, it is necessary to adjust the order of component addition and calculate the system. First, add water, buffer, and NTP, and finally add the template and enzyme.

Templates Preparation

Linearized plasmids with T7 promoters, PCR products, or synthesized DNA fragments can all serve as in vitro transcription templates for T7 High Yield RNA Transcription Kit, which can be dissolved in TE buffer or RNase free water.

1. Plasmid template (it is recommended to add 1μg linearized plasmid as the template for each reaction).
   1.1. Plasmids with T7 promoters can serve as transcription templates, and the linearization and purity of plasmids can affect transcription yield and RNA integrity.Due to the lack of effective termination, circular plasmids can transcribe RNA products of different lengths. In order to obtain specific lengths of RNA, plasmids must be completely linearized. For linearized plasmids, please ensure that the double strand has a flat end or the 5 'end has a protruding structure.
2. PCR product template (it is recommended to add 0.1μg~1μg as the template for each reaction).
   2.1. PCR products with T7 promoter can serve as in vitro transcription templates.When amplifying the template by PCR, the T7 promoter is added to the 5 'end of the upstream primer of the sense chain. The PCR product is purified and used as a template.
3. Synthetic DNA template (it is recommended to add 0.1μg~0.5μg as the template for each reaction).
   3.1. The synthesized DNA fragment with T7 promoter can also serve as a template for in vitro transcription.

Reaction system

1. Unmodified RNA system

Component	Volume
10×Transcription Buffer	2μl
ATP/GTP/CTP/UTP Mix Each (100mM)	1.5μl each
Template DNA	100ng-1μg
Enzyme Mix	1μl
RNase Free Water	Up to 20μl

2. Modified RNA system

Component	Volume
10×Transcription Buffer	2μl

Modified ATP/GTP/CTP/UTP Mix Each (100mM)	1.5μl each
Template DNA	100ng-1μg
Enzyme Mix	1μl
RNase Free Water	Up to 20μl

Modified NTP such as: pUTP, 5-Me-CTP, N1-Me-pUTP, 5-OMe-UTP etc.

3. Co transcriptional system

Component	Volume
10×Transcription Buffer	2μl
ATP/CTP/UTP Mix Each(100mM)	1.5μl each
GTP(100mM)	1.5μl
CAP1 GAG(100mM)	1.5μl
Template DNA	1μg
Enzyme Mix	1μl
RNase Free Water	Up to 20μl

Taking CAP1 GAG as an example, if using other hat structures, please refer to the recommended proportion of hat structures to prepare the reaction system

Protocol

1. In vitro transcription
   1.1. Shake and mix the components except for T7 RNA Polymerase Mix, briefly centrifuge and collect them at the bottom of the tube, and store them on ice for future use.
   1.2. Add the following components at room temperature.

Note: Calculate the system, first add water, buffer, and NTP, and finally add template and enzyme

Component	Volume
10×Transcription Buffer	2μl
ATP/GTP/CTP/UTP Mix Each	1.5μl each
Template DNA	Xμl
Enzyme Mix	1μl
RNase Free Water	Up to 20μl

   1.3. Gently mix all components with a pipette and collect them through brief centrifugation. Incubate at 37℃ for 3 hours.

*To avoid the impact of evaporation on the reaction system, it is recommended to conduct the reaction in a PCR instrument.The reaction time can be adjusted appropriately based on the size of the product fragment. For example, synthesizing RNA less than 0.3kb can extend the reaction to 4 hours or more, and the overnight reaction at 16 hours will not affect the quality of the product.

   1.4. Add 2-4U of DNase I Recombinant GMP grade to the reaction system, incubate at 37℃ for 15 minutes, and digest the transcribed DNA template. (Optional).

*Compared to the product RNA, the content of template DNA is very low and generally does not need to be removed. It can also be digested using DNaseI.

   1.5. After electrophoresis analysis and purification, the synthesized RNA can be used for downstream experiments.

*The product concentration is extremely high and needs to be diluted with RNase free water before detection.

2. Product purification.
   2.1. Phenol/chloroform purification method.

Phenol/chloroform extraction can remove proteins and most free nucleotides.

      2.1.1. Dilute the product to 180μl by adding 160μl RNase free water.
      2.1.2. Add 20μl 3M sodium acetate (pH 5.2) to the diluted product and mix thoroughly with a pipette.
      2.1.3. Add 200μl phenol/chloroform mixture (1:1) for extraction, centrifuge at 10000 rpm for 5 minutes at room

temperature, and transfer the upper solution (aqueous phase) to a new RNase free EP tube.

2.1.4. Add chloroform of the same volume as water for extraction twice and collect the upper aqueous phase.

2.1.5. Add twice the volume of anhydrous ethanol and mix well. Incubate at -20°C for at least 30 minutes, centrifuge at 15000rpm at 4°C for 15 minutes.

2.1.6. Discard the supernatant and add 500μl pre cooled 70% ethanol to wash the RNA precipitate. Centrifuge at 15000rpm at 4 °C and discard the supernatant.

2.1.7. Open the lid and dry for 2 minutes, add 20-50 μ Dissolve RNA precipitation in RNase free water or other buffer solution.

2.1.8. Storage at -80°C

2.2. Column Purification

2.2.1. Column purification can remove proteins and free nucleotides.

2.2.2. Before purification, add 80μl RNase free Water to dilute the product to 100μl, and then purify according to the column purification instructions.

*Due to the high RNA production, to avoid exceeding the carrying capacity of the binding column, please estimate the required number of columns

2.3. Magnetic bead purification

2.3.1. Magnetic bead purification can remove proteins and free nucleotides

2.3.2. Purify according to the magnetic bead purification instructions.

2.4. Lithium chloride purification

2.4.1. Add 30μl RNase Free Water and 30μl Lithium Chloride Precipitation Solution to each of the 20μl transcripts products.((Note: When RNA is less than 300nt or concentration is less than 100ng/μl, effective precipitation cannot be obtained through this method.The precipitation effect is best when the RNA concentration is greater than 400ng/μl.When the concentration of transcripts is low, between 100 and 400ng/μl, it is not necessary to dilute with water and can be precipitated with 30μl Lithium Chloride Precipitation Solution directly).

2.4.2. Mix well and place at -20°C for at least 30 minutes.

2.4.3. Centrifuge at 12000 rpm for 15 minutes, remove supernatant, and collect sediment.

2.4.4. Wash three times with pre cooled 70% ethanol.

2.4.5. Detection of RNase Free Water after re dissolution.

3. RNA quantification

3.1. UV absorption method:Free nucleotides can affect the accuracy of quantification, so please perform RNA purification before using this method.

3.2. Dye method: RiboGreen dye is used for RNA quantification, and free nucleotides do not affect quantification. It can accurately quantify RNA in purified or unpurified reaction products.

FAQ

1. How to select restriction endonucleases when linearizing plasmid templates?

Plasmids with promoters can serve as transcription templates, and the linearization and purity of plasmids can affect transcription yield and RNA integrity.Due to the lack of effective termination, circular plasmids can transcribe RNA products of different lengths. In order to obtain specific lengths of RNA, plasmids must be completely linearized, and linearized plasmids must ensure that the double strand is flat ended or the 5 'end is a protruding structure.Therefore, it is necessary to select Class II restriction endonucleases that can produce flat or 5 'terminal protruding structures, and the enzyme's recognition site is a rare site.

2. Is there a requirement for the purity of transcription templates?

The template DNA should be RNaseA Free and of high purity, with a recommended OD260/280 range of 1.8~2.0.

3. Do transcription templates need to be removed?

It is best to add DNase I Recombinant GMP grade after transcription to remove the template.

4. Low transcript production or transcription failure:

Suggest creating a control group and an experimental group. If the control group's experimental production is normal but the experimental group's production is low, there may be quality issues with the template itself leading to low production. Please try the following solutions:

4.1. There are components in the experimental template that inhibit the reaction. It is recommended to purify the template again to determine the quantification and completeness of the template;

4.2. Regarding the sequence issue of the experimental template, it is recommended to extend the reaction time at 37℃, increase the amount of template input, or try other promoters and RNA polymerase.

5. Low production of short fragment transcripts:

When the transcription product is less than 0.3kb, prolonging the reaction time or increasing the template quantity can increase RNA production.

6. Product electrophoresis tailing phenomenon:

6.1. Experimental operation process contaminated with RNase;

6.2. DNA template contaminated with RNase;

It is recommended to purify the template DNA again, and pay attention to RNase contamination control during all experimental processes.

7. RNA product fragments greater than expected:

The plasmid template is not fully linearized or the 3 'end of the sense chain is a protruding structure. It is recommended to re linearize the plasmid template to ensure complete linearization and linearized plasmids. Please ensure that the double chain is a flat end or the 5' end is a protruding structure;

RNA has a secondary structure that is not completely denatured. Replace the denatured gel to detect RNA products.

8. RNA product fragments smaller than expected:

8.1. The template sequence includes a termination sequence similar to T7 RNA polymerase, which leads to early termination of transcription. It is recommended to try replacing RNA polymerase;

8.2. Advanced structures are formed in the template, and it is recommended to try adding SSB proteins;

8.3. RNase contamination.

Related products

Product Number	Product Name
M062	Vaccinia Capping Enzyme
GMP-RI01	RNase Inhibitor, GMP Grade
M072	mRNA Cap 2´ O Methyltransferase
GMP-DI05	DNase I Recombinant GMP grade
M082	Cap 1 Capping System
M036	Pyrophosphatase, Inorganic (yeast) (ppase)
TR01	T7 RNA Polymerase
M012	Poly(A) Polymerase