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High Yield T7 RNA Transcription Kit ,CAP 1 GAG

Product Number: CA141

Shipping and Storage

-20°C

Components

Component	CA141
Enzyme Mix	20μl
10×Transcription Buffer	40μl
ATP (100 mM)	40μl
UTP (100 mM)	40μl
GTP (100 mM)	40μl
CTP (100 mM)	40μl
N1-Me-Pseudo UTP (100mM)	40μl
CAP1 GAG (100mM)	40μl
Control template (100ng/μl)	10μl
Lithium Chloride Precipitation Solution	0.75ml
DNase I, RNase-free (2U/μl)	40μl
RNase Free Water	1ml

Description

As a biomolecule, mRNA can be synthesized on a large scale through IVT, in vitro transcription. The T7 promoter is currently one of the most efficient promoters, so using 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 transcriptional 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 co transcription kit can use DNA containing the T7 promoter sequence, AG start sequence, and poly (A) tail coding region as templates, and incorporate CAP1 GAG cap analogues during transcription to obtain mRNA with 5'-Cap and 3'-poly (A) tails. The 5'-Cap structure can prevent mRNA from being cleaved by nucleases, thereby maintaining mRNA stability and initiating translation, and improving mRNA stability in vivo or within cells Translation efficiency and immunogenicity have a significant impact.

This reagent kit can transcribe 150-200μg of RNA in one reaction, and the synthesized RNA can be used for downstream applications such as microinjection, transfection, and in vitro translation experiments.

Application

Synthesize single stranded RNA with CAP1 GAG structure, which can be used as a substitute for other cap analogues.

Note

1. Template efficiency and incubation time:

   This kit can produce 150-200μg of RNA with a template input of 1μg. However, the yield of different templates may vary depending on the sequence, structure, length, purity of the templates, as well as the sequence and length of specific RNA polymerase promoters. The pollutants that affect transcription yield include ribonucleases or pollutants such as phenol, trace metals, and SDS.

2. Optimized response:

   The recommended reaction conditions can be applied to in vitro transcription of most templates, but for some templates, the

yield can be improved by extending the reaction time (4 hours overnight reaction) and increasing the amount of templates used.

3. Maintain RNase free environment:
   Use RNase free tubes and pipettes;
   When handling kit components or samples containing RNA, gloves should be worn and replaced frequently, especially when in contact with potential sources of RNase contamination, such as door handles, pens, pencils, and human skin.
   When not in use, all reagents should be sealed properly. During the incubation process, seal all test tubes containing RNA.
4. Due to the presence of spermidine components in the 10 Transcription Buffer, it will precipitate with the template DNA at low temperatures. When preparing the reaction solution, it is necessary to do so at room temperature. Adjust the order of component addition and calculate the system. First, add water, buffer, and NTP, and finally add the template and enzyme. The buffer should not be stored at room temperature for a long time. The optimal storage temperature is -20 ℃. When taking it out for use, dissolve it thoroughly and mix well before use.

Template preparation

Linearized plasmids, PCR products, or synthesized DNA fragments with T7 promoter and AG starting sequence can serve as High Yield T7 RNA Transcription Kit, CAP 1 GAG transcription templates, which can be dissolved in TE buffer or RNase free water.

1. Plasmid template (it is recommended to add 1μg of linearized plasmid as a template for each reaction)
   Plasmids with T7 promoter and AG starting sequence can serve as transcription templates, and plasmid linearization and purity 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 is a protruding structure.
2. PCR product template (it is recommended to add 0.1μg~1μg as a template for each reaction)
   PCR products with T7 promoter and AG starting sequence can serve as in vitro transcription templates. When amplifying the PCR template, 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 a template for each reaction)
   The synthesized DNA fragments with T7 promoter and AG starting sequence can also serve as templates for in vitro transcription.

Protocol

1. In vitro transcription

1.1. Shake and mix all components except for Enzyme Mix, centrifuge briefly and collect at the bottom of the tube. Store on ice for later 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.

Reagent	Unmodified system
10×Transcription Buffer	2μl
ATP/GTP/CTP/UTP (100mM Each)	1.5-2μl each
CAP1 GAG (100mM)	1.5-2μl
Template DNA	500ng-1μg
Enzyme Mix	1μl
RNase Free Water	Up to 20μl
Reagent	**Modification system**
10×Transcription Buffer	2μl
ATP/GTP/CTP (100mM Each)	1.5-2μl each
N1-Me-Pseudo UTP (100mM)	1.5-2μl
CAP1 GAG (100mM)	1.5-2μl

Template DNA	500ng-1μg
Enzyme Mix	1μl
RNase Free Water	Up to 20μl

Note: If other hat analogs are used, the ratio of GTP to hat analogs should be adjusted according to the specific hat analogs, and the recommended ratio is between 4:1 and 1:1.

1.3. Gently mix each component with a pipette and collect by centrifugation briefly. Incubate at 37 °C 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 according to the size of the product fragment. For example, when synthesizing RNA smaller than 0.3 kb, the reaction can be extended to 4 hours or longer. Overnight reaction after 16 hours will not affect the quality of the product.

1.4. Add 2-4U of DNase I to the reaction system, incubate at 37 °C 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. Lithium chloride purification

2.1.1. Add 30μl of Lithium Chloride Precision Solution (7.5 M Lithium Chloride, 50 mM EDTA) and 30μl of RNase Free Water (note: effective precipitation cannot be obtained when RNA concentration is less than 300nt or 100ng/μl) to a volume of 20μl. The best precipitation effect is achieved when RNA concentration is greater than 400ng/μl. When the concentration of transcription products is low, between 100-400ng/μl, there is no need to dilute with water. Instead, use 30μl Lithium Chloride Precision Solution for precipitation;

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

2.1.3. Centrifuge at 12000 rpm for 15 minutes, remove the supernatant, and collect the precipitate.

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

2.1.5. Detection after RNase Free Water redissolution

2.2. Magnetic bead purification

2.2.1. Magnetic bead purification can remove proteins and free nucleotides.

2.2.2. Purify according to the magnetic bead purification instructions.

2.3. Column purification

2.3.1. Column purification can remove proteins and free nucleotides.

2.3.2. Dilute the product to 100μl by adding 80μl of RNase free water before purification, 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.

2.4. Phenol/chloroform purification method

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

2.4.1. Dilute the product to 180μl by adding 160μl of RNase free water.

2.4.2. Add 20μl of 3M sodium acetate (pH 5.2) to the diluted product and mix thoroughly with a pipette.

2.4.3. Add 200μl of 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

2.4.4. Add chloroform of equal volume to water for extraction twice and collect the upper aqueous phase.

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

2.4.6. Discard the supernatant and wash the RNA precipitate with 500μl of pre cooled 70% ethanol. Centrifuge at 4 °C

and 15000 rpm to discard the supernatant.

2.4.7. Open the lid and dry for 2 minutes, then add 20-50μl of RNase free water or other buffer to dissolve the RNA precipitate.

2.4.8. -Store at 80°C.

3. **RNA quantification**

3.1. UV absorption method: Free nucleotides can affect the accuracy of quantification. 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 plasmid linearization and purity 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. Linearized plasmids must ensure that the double strand is flat or the 5'end is a protruding structure. At the same time, in order to ensure that there are no excess base sequences in the linear template after enzyme digestion, it is necessary to select IIS type restriction endonucleases that can produce flat or 5'end protruding structures, such as BsaI (product code: M062012), BspQI (product code: M062011), etc., and the enzyme's recognition sites are rare sites.

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 of 1.8-2.0.

3. Do transcription templates need to be removed?

It is best to add DNase I to remove the template after transcription is complete.

4. Low transcript production or transcription failure:

Suggest creating a control group and an experimental group. If the production of the control group is low, please contact us. If the control group's experimental yield is normal but the experimental group's yield is low, there may be quality issues with the template itself leading to low yield. 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 issue with the experimental template sequence, it is recommended to extend the reaction time at 37 °C, increase the amount of template input, or try other promoters and RNA polymerase;

4.3. The transcription buffer is not fully dissolved. It is recommended to completely dissolve the buffer and mix well before use.

5. Low production of short transcription products:

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

6. Product electrophoresis tailing phenomenon:

6.1. The experimental operation process was contaminated with RNase;

6.2. DNA template contaminated with RNase;

Suggest re purifying the template DNA and paying attention to RNase contamination control during all experimental processes.

7. RNA product fragments greater than expected:

7.1. The plasmid template is not completely 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. For linearized plasmids, please ensure that the double chain is a flat end or the 5' end is a protruding structure;

7.2. 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 the RNA polymerase;
   - 8.2. Form advanced structures in the template, it is recommended to try adding SSB protein;
   - 8.3. RNase contamination.
9. How to measure the capping rate of transcripts.

   It is recommended to use LC-MS instrument for determining the capping rate.

Related products

Product Number	Product Name
M072	mRNA Cap 2' O Methyltransferase
CP082	Cap 1 Capping System Kit
E131	T7 High Yield RNA Transcription kit
GMP-RI01	RNase Inhibitor, GMP Grade
GMP-DI05	DNase I Recombinant GMP grade
GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade
GMP-M012	Poly(A) Polymerase, GMP Grade