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Low dsRNA High Yield T7 RNA Co-Transcription Kit, with CAP 1 GAG

Product Number: CA341

Shipping and Storage

Store at -20℃±5℃.

Component

Component	CA341-20T
Enzyme Mix 2.0	20μL
10×Transcription Buffer-A	40μL
10×Transcription Buffer-B	40μL
ATP (200mM Tris Solution)	20μL
UTP (200mM Tris Solution)	20μL
GTP (200mM Tris Solution)	20μL
CTP (200mM Tris Solution)	20μL
N1-Me-Pseudo UTP (200mM Tris Solution)	20μL
CAP1 GAG (100mM)	40μL
Control template (100ng/μL)	20μL
Lithium Chloride Precipitation Solution	1mL
DNase I, RNase-free (2U/μL)	40μL
RNase Free Water	1mL

Description

As a biomacromolecule, mRNA can be synthesized on a large scale through in vitro transcription (IVT). The T7 promoter is currently one of the most efficient promoters for transcription. Therefore, using T7 RNA polymerase (T7 RNA polymerase) for in vitro transcription can easily and quickly obtain a large number of RNA molecules. This kit optimizes the co transcription reaction system and uses T7 RNA polymerase 2.0, a T7 RNA polymerase mutated enzyme obtained through molecular evolution, which can significantly reduce the production of dsRNA during transcription.

This co transcription kit can use DNA containing T7 promoter sequence, AG promoter sequence, and poly (A) tail coding region as templates, and incorporate CAP1 GAG cap analogs during transcription to obtain mRNA with 5 '- Cap1 and 3' - poly (A) tails. The 5 '- Cap1 structure can prevent mRNA from being degraded by nucleases, thereby maintaining mRNA stability and initiating translation, which has a significant impact on mRNA stability, translation efficiency, and immunogenicity in vivo or in cells.

This reagent kit can transcribe 150-250μg of RNA in one reaction, and the co transcription cap rate is greater than 95%. The synthesized RNA can be used for research on RNA structure and function, RNA enzyme protection, probe hybridization, etc RNAi、Downstream applications such as microinjection and in vitro translation.

Application

Synthesize single stranded RNA with CAP1 GAG structure, which can be replaced with other cap analogs for use.

Note

1. Template efficiency and incubation time: This kit can produce 150-250μg 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 template, as well as the sequence and length of specific RNA polymerase promoters. Pollutants that affect transcription yield include ribonucleases or

contaminants such as phenol, trace metals, and SDS.

2. Optimized reaction: The recommended reaction conditions can be applied to the in vitro transcription of most templates, but for some templates, the yield can be improved by extending the reaction time (4-hour overnight reaction) and increasing the amount of template used.
3. Maintain RNase free environment: use RNase free tubes and pipettes; Gloves should be worn when handling kit components or samples containing RNA, and gloves should be changed 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 in the 10 × Transcription Buffer-A/B, it will form a precipitate with the template DNA at low temperatures. When preparing the reaction solution, it needs to be done at room temperature. Adjust the order of component addition, calculate the system, add water, buffer, and NTP first, and finally add the template and enzyme. Do not store this buffer 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.

Templates preparation

Linearized plasmids, PCR products, or synthesized DNA fragments with T7 promoter and AG start sequence can be used as T7 High Yield RNA Co Transcription Kit for Low dsRNA with CAP1 GAG transcription templates, which can be dissolved in TE buffer or RNase free water.

1. Plasmid template (it is recommended to add 1μg linearized plasmid as a template for each reaction)

Plasmids with T7 promoter and AG initiation sequence 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 RNA of a specific length, the plasmid must be completely linearized. Linearized plasmids should ensure that the double stranded end is flat or the 5 'end is a protruding structure.

2. PCR product template (recommended to add 0.1μg~1μg as a template for each reaction)

PCR products with T7 promoter and AG initiation sequence can be used as in vitro transcription templates. Add the T7 promoter to the 5 'end of the upstream primer of the sense chain during PCR amplification of the template. The PCR product is purified and used as a template.

3. Synthetic DNA template (recommended to add 0.1μg~0.5μg as template for each reaction)

The synthesized DNA fragments with T7 promoter and AG initiation sequence can also serve as templates for in vitro transcription.

Protocol

1. In vitro transcription

1.1. Dissolve 10 × Transcription Buffer-A, NTPs, and CAP1 GAG, centrifuge briefly, and collect at the bottom of the tube. Store the remaining materials 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 the template and enzyme.

Component	Non modified system
10×Transcription Buffer-A	2μL
ATP/GTP/CTP/UTP (Each 200mM)	1μL each
CAP1 GAG (100mM)	1.5-2μL
Enzyme Mix 2.0	1μL
Template DNA	1μg
RNase Free Water Up to	20μL

Component	Non modified system
10×Transcription Buffer-A	2μL

ATP/GTP/CTP (Each 200mM)	1μL each
N1-Me-Pseudo UTP (200mM)	1μL
CAP1 GAG (100mM)	1.5-2μL
Enzyme Mix 2.0	1μL
Template DNA	1μg
RNase Free Water Up to	20μL

Note: When using 10 × Transcription Buffer-A in a co transcription system, if the transcription yield is low, 10 × Transcription Buffer-B can be used instead.

If other cap analogues are used, the ratio of NTP to cap analogues should be adjusted according to the specific cap analogue, and the recommended ratio is between 4:1 and 1:1. If you see a white precipitate after the reaction is complete, it is magnesium pyrophosphate produced during the reaction and will not affect subsequent experiments. You can choose to add some EDTA to remove it or recover the supernatant by centrifugation.

1.3. Gently mix the components with a pipette and collect them by centrifugation briefly. Incubate at 37℃ for 2-3 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 0.3kb, the reaction can be extended to 4 hours or longer. Overnight reaction for 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℃ 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 with DNaseI.

1.5. The synthesized RNA can be used for downstream experiments after electrophoresis analysis and purification.

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

2. Product purification

2.1. Purification of lithium chloride

2.1.1. Add 30μL of Lithium Chloride Preparation Solution (7.5 M Lithium Chloride, 50 mM EDTA) and 30μL of RNase Free Water (note: effective precipitation cannot be obtained by this method when RNA is less than 300nt or concentration is less than 100ng/μL) to a volume of 20μL. The precipitation effect is best when the 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, and 30μL Lithium Chloride Precipitation Solution can be directly used for precipitation;

2.1.2. Mix well and place at -20℃ for 30 minutes.

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

2.1.4. Wash three times with pre cooled 75% ethanol.

2.1.5. Detection after RNase Free Water reconstitution.

2.2. Magnetic bead purification

Magnetic bead purification can remove proteins and free nucleotides.

Purify according to the instructions for magnetic bead purification.

2.3. Column Purification

Column purification can remove proteins and free nucleotides.

Dilute the product to 100μL by adding 80μL RNase free water before purification, and then purify according to the column purification instructions.

Due to the high RNA production, in order 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 with an equal volume to water for extraction twice, and collect the upper aqueous phase.

2.4.5. Add 2 times the volume of anhydrous ethanol and mix well. Incubate at -20℃ for at least 30 minutes and centrifuge at 15000 rpm at 4℃ for 15 minutes.

2.4.6. Discard the supernatant and add 500μL of pre cooled 70% ethanol to wash the RNA precipitate. Centrifuge at 15000 rpm at 4℃ and discard the supernatant.

2.4.7. Open the lid and dry for 2 minutes. Add 20-50μL of RNase free water or other buffer solution to dissolve the RNA precipitate.

2.4.8. Store at -80℃.

3. RNA quantification

UV absorption method: Free nucleotides can affect the accuracy of quantification. Please perform RNA purification before using this method.

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 choose 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 RNA of specific lengths, the plasmid must be completely linearized, and the linearized plasmid must ensure that the double stranded end is flat or the 5 'end is a protruding structure. At the same time, in order to ensure that the linear template after enzyme digestion has no excess base sequences, it is necessary to select IIS type restriction endonucleases that can produce prominent structures at the end or 5 'end, such as BsaI, BspQI, etc., and the recognition site of the enzyme 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 of 1.8-2.0.

3. Do transcription templates need to be removed?

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

4. Low transcript production or transcription failure:

Suggest creating a control group and an experimental group. If the yield of the control group experiment is normal but the yield of the experimental group is low, there may be a quality problem with the template itself that leads to the low yield. Please try the following solution to solve it:

4.1. The experimental template contains components that inhibit the reaction. It is recommended to purify the template again to determine the quantification and integrity of the template;

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

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

4.4. When using 10 × Transcription Buffer-A with low yield, it is recommended to switch to 10 × Transcription Buffer-B for transcription.

5. Low yield 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:

The experimental operation process was contaminated by RNase;

6.1. DNA template contaminated by RNase;

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

7. RNA product fragment larger than expected:

The plasmid template is not fully linearized or has a prominent structure at the 3 'end of the sense strand. It is recommended to re linearize the plasmid template to ensure complete linearization. For linearized plasmids, please ensure that the double strand has a flat end or a prominent structure at the 5' end;

RNA has an incompletely denatured secondary structure, and the denatured gel is replaced to detect RNA products.

8. The RNA product fragment is smaller than expected:

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

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

8.3. RNase contamination.

9. How to determine the capping rate of transcription products:

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