HyperScribe™ Co-transcription mRNA Synthesis Kit Plus (EZ Cap Reagent AG (3' OMe), T7)

Introductions

HyperScribe™ Co-transcription mRNA Synthesis Kit plus (EZ Cap Reagent AG (3' OMe), T7) is designed for quick production of Cap 1 capped mRNA in vitro. Capped mRNA is synthesized by co-transcriptional incorporation of capping reagent (EZ Cap Reagent AG (3' OMe), Catalog No. B8178) using T7 RNA Polymerase. The capping of EZ Cap Reagent AG (3' OMe) results in a Cap 1 structure and ensures high translation efficacy. In addition, Polyadenylation (i.e. addition of Poly(A) tail) plays an important role in the stabilization of RNA in eukaryotes and enhances the efficiency of translation initiation. This kit recommends using a DNA template with a tailed sequence at the 3' end for producting mRNA with stable poly(A) tail (typically 100-120 A).

RNA synthesized using this kit has many applications in biological experiments, such as in vitro translation, antisense RNA and RNAi experiments, RNA vaccines, RNA structure and function studies, ribozyme biochemistry, RNase protein experiments and probe-based hybridization blots.

The kit contains sufficient reagents to carry out 25 reactions, 20 μL each time. It adjusts the composition and reaction based on K1066 to achieve higher RNA yields for standard 20 μL reaction systems.

Materials

1. Components in the kit

(1) Capped RNA Synthesis with EZ Cap Reagent AG (3' OMe)

Components	25 rxn
T7 RNA Polymerase Mix	50 μL
10 × Reaction Buffer	50 μL
ATP (100 mM)	30 μL
UTP (100 mM)	25 μL
CTP (100 mM)	25 μL

GTP (100 mM)	25 μL
EZ Cap Reagent AG (3' OMe) (100 mM)	20 μL
Control Template (0.5 μg/μL)	5 μL
RNase-free H_2O	0.5 mL
Store the components at -20°C.	

2. Materials not supplied

DNA Template

The DNA Template can be plasmid DNA, oligonucleotides, PCR products, cDNA and so on. The DNA template must be linear and contain a T7 RNA polymerase promoter sequence that determines the transcriptional start position of the target sequence. The DNA template itself can carry a poly(A) tail sequence, or the tail can be introduced by primer design. Our company can offer Biotin-NTP, Fluorescein-NTP, Digoxigenin-NTP, Aminoallyl-NTP, ARCA(B8175), Pseudo-UTP (B7972), 5mCTP(B7967), mCAP(B8174) and 5-Methoxy-UTP(B8061). For more reagents related to RNA synthesis in vitro, please refer to our website.

Protocols

1. DNA Template Preparation

PCR DNA product, linearized plasmid DNA, cDNA and oligonucleotides can be used as templates for *in vitro* transcription. Many cloning vectors carry two opposite T7 phage polymerase promoter sequences that bind T7 polymerase to initiate the transcription process. To obtain a purified linearized plasmid, the plasmid as a transcription template by digestion with restriction endonuclease treatment must be cleaned up. Figure 1 interpret how the T7 RNA Polymerase transcript to produce RNA with T7 promoter.

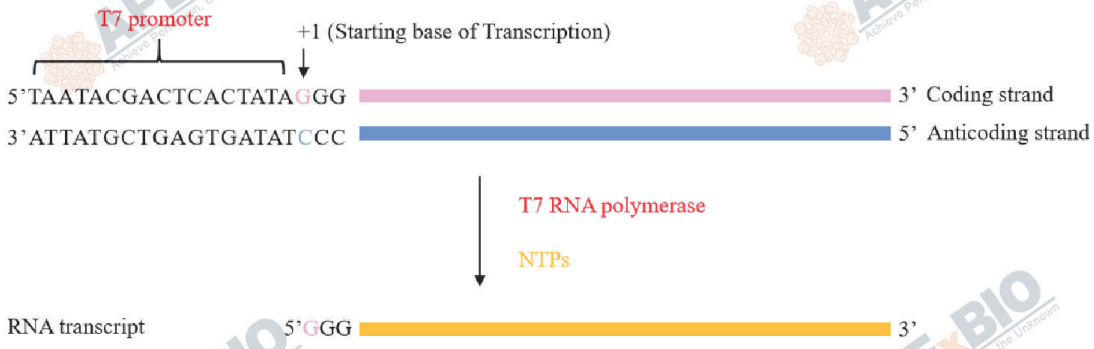

Figure 1: Transcription using T7 RNA Polymerase

- Plasmid Templates

Many plasmid cloning vectors carry two phage polymerase promoters in different directions, one on each side of the multiple cloning sites, allowing transcription of any strand of the inserted sequence. Such double reverse promoter vectors include pDP vector (Ambion), pGEM vector (Promega), pBluescript vector (Stratagene), pCRII

vector (Invitrogen) and so on. The plasmid vector used as a transcription template must be linearized by restriction endonuclease digestion. Since the transcription reaction continues until the end of the DNA template, linearization ensures to obtain RNA transcripts of the defined length and sequences. Many plasmid vectors themselves carry tailed sequences that can be linearized as transcription templates to obtain mRNA with Poly(A) tails, as shown in Figure 2. Restriction sites are not necessarily unique and as long as the promoter is kept adjacent to the transcription template, the vector itself can be digested repeatedly. Purification should be carried out after restriction enzyme digestion, as residues in the digestion reaction may inhibit the transcription reaction.

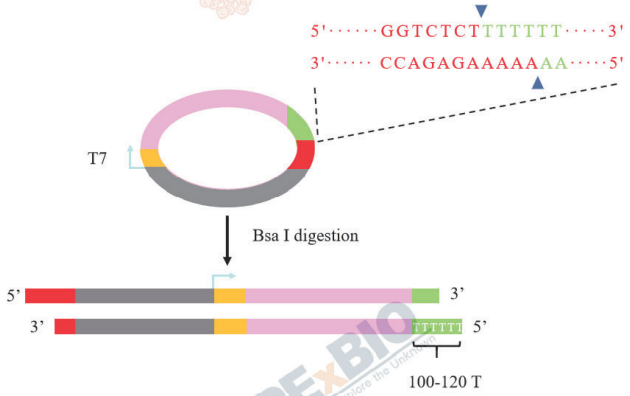

Figure 2: Plasmid digestion to obtain a transcription template with tailed sequence

■ PCR products

The PCR product can also be used as a transcription template for *in vitro* transcription. PCR products with a T7 promoter and tailed sequences can be obtained by upstream or downstream PCR primer. Figure 3 interpret how to add T7 promoter and poly(A) to PCR products.

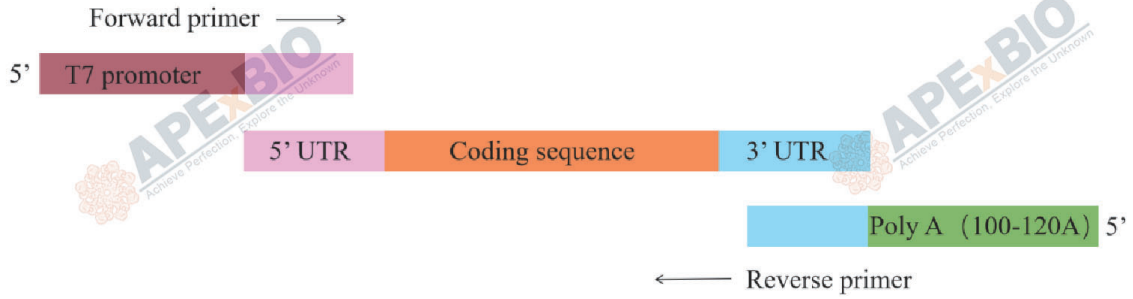

Figure 3: PCR primer design for T7 RNA Polymerase template

■ Synthetic DNA Oligonucleotides

Two oligonucleotides can also be used to construct short transcription templates. A double-stranded DNA template can be formed by simply annealing the two complementary oligonucleotides carrying the phage promoter sequence. In fact, as long as part of the DNA templates form a double-stranded DNA; the *in vitro* RNA transcriptional experiment can be performed.

■ cDNA

In recent years, RNA transcription *in vitro* procedures have been gradually applied to RNA amplification reactions:

the oligo(dT)-T7 promoter primers can be used in the reverse transcription process to obtain a transcription template using RNA as an initial template. A double-stranded transcription template will be acquired by a second strand synthesis reaction.

2. Capped RNA Synthesis

(1) Thaw corresponding components on ice.

(2) Assemble the reaction system at room temperature in the following order.

Reagent	Volume	Final Concentration
Nuclease-free water	X μL	
10 x Reaction Buffer	2 μL	
ATP (100 mM)	1.2 μL	6 mM final
UTP (100 mM)	1 μL	5 mM final
CTP (100 mM)	1 μL	5 mM final
GTP (100 mM)	1 μL	5 mM final
EZ Cap Reagent AG (3' OMe) (100 mM)	0.8 μL	4 mM final
Template DNA	X μL	1 μg
T7 RNA Polymerase Mix	2 μL	
Total Reaction Volume	20 μL	

(3) Mix thoroughly. Incubate for 2 hours at 37°C. For short (< 300nt) transcripts incubate for 4 hours at 37°C.

(4) To remove template DNA, add 2 μL of DNase I (RNase-free), mix and incubate at 37°C for 15 minutes.

Notes

1. Low Yield of Full-length RNA

If the transcription of the template produces full-length RNA, but the yield is significantly lower than expected, it may be that the contaminants in the DNA template inhibit the activity of the RNA polymerase, or the DNA concentration may not be correct. Alternatively, DNA templates may require additional purification. It is recommended to use phenol chloroform extraction (see template DNA preparation section).

2. Low Yield of Short Transcript

Short transcripts (<0.3 kb) of high yields can be obtained by extending the incubation time and increasing the amount of the template. The incubation reaction for up to 16 hours (overnight) or the use of up to 2 μg of a template will be beneficial to achieve maximum yield.

3. RNA Transcript Smearing on Denaturing Gel

If the RNA begins to appear on denaturing polyacrylamide or agarose gel (e.g. smeared), this means that RNase contaminates the DNA template. DNA templates contaminated with RNase affect the length and yield of the synthesized transcript (lower than the expected transcript length). Before processing the plasmid DNA template with the T7 High Yield RNA Synthesis Kit, we recommend using the RNase contamination assay kit to assess the quality of the plasmid DNA template. If the plasmid DNA template is contaminated with RNase, it is necessary to extract with phenol/chloroform, then precipitate the DNA and dissolve the DNA in nuclease-free water (see template DNA preparation section).

4. RNA Transcript of Larger Size than Expected

If the yield of the RNA transcript appears to be larger than expected on the denaturing gel, the plasmid template DNA may not be fully digested. Even a small amount of undigested circular DNA can produce a large number of long transcripts. Check that the template is completely digested, if the plasmid is not completely digested, restriction digestion should be repeated. Larger bands can also be observed when the RNA transcript is not completely denatured due to the presence of a stronger secondary structure.

5. RNA Transcript of Smaller Size than Expected

If the denaturation gel analysis indicates a smaller band than expected, it is most likely due to early termination of the polymerase. Some sequences similar to the T7 RNA polymerase termination signal will result in early termination of the RNA transcription reaction. Incubating the transcription reaction at a lower temperature (e.g.at 30°C) may increase the content of the full-length transcript, but the yield will decrease. Incubation at 42°C may increase the yield of full-length transcripts for GC-rich templates or templates with secondary structures.

If the transcription process is prematurely terminated in the synthesis of highly specific active radiolabeled RNA probes, the concentration of "restricted NTP" should be increased. Additional "cold" NTPs can be added to the reaction to increase the proportion of full-length transcripts, however an increase in the yield of the full-length product will compromise the specific activity of the probe.

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