HyperScribe™ SP6 High Yield RNA Synthesis Kit

Introductions

HyperScribe™ SP6 High Yield RNA Synthesis Kit performs *in vitro* RNA transcription experiment in a relatively short time using SP6 RNA polymerase. The kit is designed for high yields of RNA transcripts, highly specific radiolabeled RNA probes and to obtain capped, dye-labeled or biotinylated RNA incorporated modified nucleotides.

RNA synthesized using this kit covers a wide range of applications, 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 25/50/100 reactions, 20 μL each time. ≥50 μg of RNA can be generated with 1 μg of control template per standard reaction.

Materials

1. Components and storage

Components	25 rxns	50 rxns	100 rxns
SP6 RNA Polymerase Mix	50 μL	100 μL	200 μL
10 × Reaction Buffer	50 μL	100 μL	200 μL
ATP (100 mM)	25 μL	50 μL	100 μL
GTP (100 mM)	25 μL	50 μL	100 μL
UTP (100 mM)	25 μL	50 μL	100 μL
CTP (100 mM)	25 μL	50 μL	100 μL
Control Template (0.5 μg/μL)	5 μL	10 μL	20 μL
RNase-free H_2O	0.5 mL	1 mL	2×1 mL
DNase I（RNase-free）(2 U/μl）	25 μL	50 μL	100 μL

Store all the kit 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 SP6 RNA polymerase promoter sequence that determines the transcriptional start position of the target sequence. 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 polymerase promoter sequences that bind RNA 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 SP6 RNA Polymerase transcript to produce RNA with SP6 promoter.

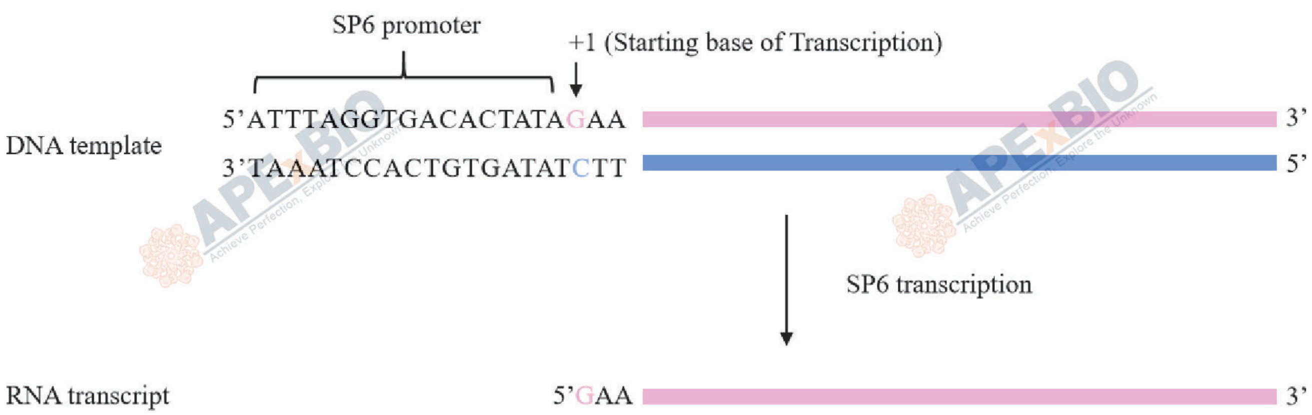

Figure 1: Transcription using SP6 RNA Polymerase

1.1. Plasmid Templates

Many plasmid cloning vectors carry two phage polymerase promoters in different directions, one on each side of the multiple cloning site, 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. 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.

After linearization, we recommend purifying the template DNA by phenol/chloroform extraction:

1. Extract DNA with an equal volume of 1:1 phenol/chloroform mixture, repeat if necessary.

2. Extract twice with an equal volume of chloroform to remove residual phenol.

3. Precipitate the DNA by adding 1/10th volume of 3 M sodium acetate, pH 5.2, and two volumes of ethanol. Incubate at –20°C for at least 30 minutes.

4. Pellet the DNA in a microcentrifuge for 15 minutes at top speed. Carefully remove the supernatant.

5. Rinse the pellet by adding 500 µL of 70% ethanol and centrifuging for 15 minutes at top speed. Carefully remove the supernatant.

6. Air dry the pellet and resuspend it in nuclease-free water at a concentration of 0.5–1 µg/µL.

1.2. PCR products

The PCR product can also be used as a transcription template for *in vitro* transcription. PCR products with a SP6 promoter can be obtained by adding a SP6 promoter sequence to the 5' end of the upstream or downstream PCR primer. These sequences form a double-stranded product with a promoter sequence by PCR reaction.

1.3. 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.

1.4. cDNA

In recent years, RNA transcription *in vitro* procedures have been gradually applied to aRNA amplification reactions: the oligo(dT)-SP6 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. RNA Synthesis

(1) Thaw corresponding components on ice.

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

(Note: components must be mixed at room temperature).

Nuclease-free water	X μL	
10xReaction Buffer	2 μL	
ATP (100 mM)	1 μL	5 mM final
GTP (100 mM)	1 μL	5 mM final
UTP (100 mM)	1 μL	5 mM final
CTP (100 mM)	1 μL	5 mM final
Template DNA	X μL	1 μg
SP6 RNA Polymerase Mix	2 μL	
Total Reaction Volume	20 μL	

(3) Mix thoroughly. Incubate for 2 h at 37°C. Incubate the transcripts of short fragments (<300nt) for 4 h.

(4) (Optional) Add 1μL of DNase I to the reaction system and incubate at 37°C for 15min to digest the DNA template. Compared with the product RNA, the content of template DNA is very low. Generally, it does not need to be removed, and it can also be digested with DNase I.

(5) Continue to purify the synthesized RNA or detect the transcription product by gel electrophoresis. Figure 2 illustrates the effect of template amount on RNA yield.

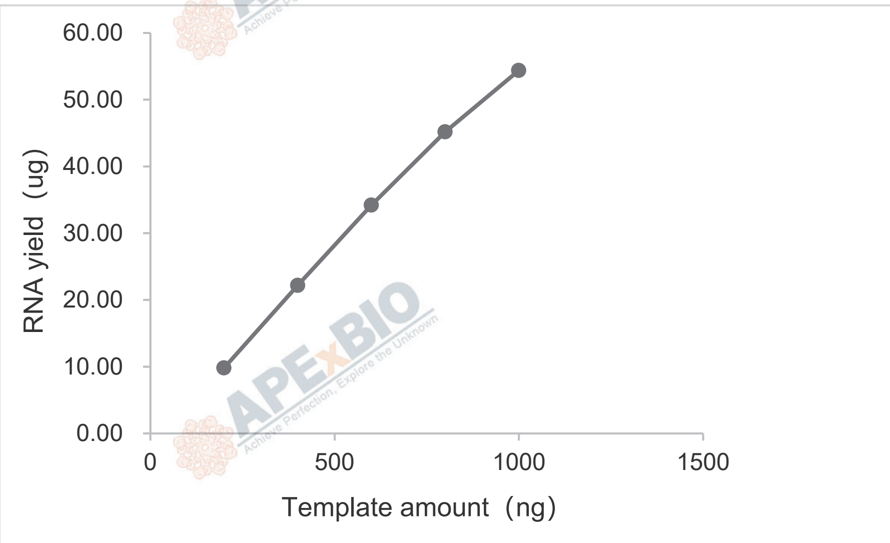

Figure 2: The effect of template amount on RNA yield.

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 degraded (e.g. smeared) 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 SP6 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 SP6 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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