Manual

AmpliScribe T7 High Yield Transcription Kit

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1. Introduction

The AmpliScribe T7 High Yield Transcription Kit is specially formulated to enable users to obtain the maximum possible yields of RNA from an *in vitro* transcription reaction. The standard 2 hour, 20 µL AmpliScribe reaction will incorporate up to 90% of input NTPs and produce up to 150 µg of RNA from 1 µg of the control template. These yields are made possible by the high-performance properties of the AmpliScribe T7 enzyme preparations.

AmpliScribe Kits produce exceptionally high yields of either long or short transcripts. The standard AmpliScribe reaction can be scaled up to produce milligram amounts of RNA. In addition, an AmpliScribe reaction can be readily modified to prepare fluorescent[1]-, biotinylated[2]-, or digoxigenin[3]-labeled RNA.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
AmpliScribe T7 High Yield Transcription Kit	50 Rxns	AS3107	AmpliScribe T7 RNA Polymerase	E0006NI-D	100 µL
			AmpliScribe T7 10X Reaction Buffer	SS000019-D3	200 µL
			ATP (100 mM)	SS000210-D3	100 µL
			GTP (100 mM)	SS000207-D3	100 µL
			CTP (100 mM)	SS000208-D3	100 µL
			UTP (100 mM)	SS000209-D3	100 µL
			DTT (100 mM)	SS000065-D5	125 µL
			RiboGuard™ RNase Inhibitor (40 U/µL)	E0126-40D5	30 µL
			Nuclease-Free Water, Sterile	SS000772-D3	1.0 mL
			Control Template DNA (0.5 µg/µL)	SS000571-D2	25 µL
			RNase-Free DNase I (1 U/µL)	E0013-1D7	60 µL

3. Product specifications

Storage: Store only at -20 °C in a freezer without a defrost cycle. Do not store at -70 °C.

Contaminating Activity Assays: All of the components of the AmpliScribe Kits are free of detectable RNase activity, and all of the components except DNase I are free of detectable exonuclease and endonuclease activities.

Control Template: The control template is a 4.2 kb linearised plasmid, containing a 1.4 kb lambda DNA insert that will produce a 1,380-b runoff transcript.

RNase-Free DNase I Unit Definition: One Molecular Biology Unit (MBU) of RNase-Free DNase I digests 1 µg of pUC19 DNA to oligodeoxynucleotides in 10 minutes at 37 °C.

4. Notes on using the AmpliScribe Kits

1. Template preparation: Transcription templates should be linear, double-stranded DNA with blunt or 5′-protruding ends. Templates containing 3′-protruding ends can produce spurious transcripts due to non-specific initiation. PCR products and cDNA can also be used as templates, provided that the appropriate promoter has been incorporated into one of the primers used.

   The quality of the DNA template directly affects the quantity and quality of the RNA produced. Generally, DNA is of sufficient quality for use if it is free of contaminating RNases and can be completely digested with restriction enzymes. To confirm that a template is fully linearised and intact, examine the DNA on an ethidium-stained agarose or polyacrylamide gel prior to use.

   Templates that give low yields or less than full-length transcripts may contain RNases or other contaminants. Such templates will usually give better results after the following treatment:

   a) Add Proteinase K to 100-200 µg/mL and SDS to 0.5% final concentrations.
   b) Incubate for 30-60 minutes at 37 °C.
   c) Extract with an equal volume of a 1:1 mixture of TE-saturated phenol/chloroform.
   d) Ethanol-precipitate.
   e) Gently remove the supernatant and rinse the pellet with 70% ethanol.
   f) Resuspend at 1.0 µg/µL in RNase-Free TE (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

2. Template efficiency: Linearised plasmid templates and PCR product templates that produce transcripts of equivalent sizes are utilised with equal efficiency by the AmpliScribe Kits. The T7 Control DNA Template produces 150 µg of a ~1.4 kb RNA per 1 µg of DNA template in a standard 20 µL, AmpliScribe T7 reaction. Different templates may give different yields. Lower yields from an experimental template could be due to:

   a) *Quality of template prep:* Degraded templates, RNases, or contaminants such as phenol, trace metals, and SDS may reduce yields.
   b) *Transcriptional efficiency:* Different templates may be transcribed more or less efficiently based on promoter strength, reinitiation rate, and termination efficiency.
   c) *Size of the template:* Yields may also differ based on the size of the template.

Amount of control template	Yield of RNA
1,000 ng (1 µg)	130-160 µg
500 ng (0.5 µg)	110-130 µg
100 ng (0.1 µg)	30-50 µg
50 ng (0.05 µg)	15-25 µg
10 ng (0.01 µg)	10-20 µg
1 ng (0.001 µg)	3-8 µg

Table 1. Yield of RNA from reduced amounts of control template DNA from a standard 20 µL, 2-hour AmpliScribe T7 High Yield Transcription Reaction. Results may vary depending on the template.

3. **Amount of template:** The standard 20 µL, 2-hour AmpliScribe reaction is optimised for transcription using 1 µg of linearised DNA template; however, lower amounts of DNA template can be used successfully. Table 1 summarises our experiences with reducing the amount of control DNA template in a standard AmpliScribe T7 reaction. Results may vary depending on the template used. Increasing the reaction time to 4-6 hours may increase the yield of RNA.
4. **Reaction assembly:** Assemble an AmpliScribe reaction at room temperature. Assembly of the reaction at temperatures lower than 22 °C can result in formation of an insoluble precipitate. Storing the AmpliScribe T7 10X Reaction Buffer at -70 °C may result in the formation of a white precipitate. If this happens, heat the tube to 37 °C for 5 minutes and mix thoroughly to resuspend the precipitate.
5. **Optimising the reaction:** The recommended reaction conditions should give excellent results with most templates. Modifying the protocol may, however, improve results with some templates. One way to increase yield is to extend the incubation to 4-6 hours. A second way to increase yield in some cases is to raise the template concentration. Finally, increasing the reaction temperature from 37 °C to 42 °C may also improve the yield.
6. **Yield of "short" RNA transcripts:** Although the number of micrograms of short RNA produced in a standard AmpliScribe reaction is small compared to the yield of long transcripts, the number of moles of short RNA produced is often equal to the number of moles of long RNA produced.

5. Standard AmpliScribe high yield transcription reaction

For use with the AmpliScribe T7 RNA Polymerase

Important! Combine the following reaction components at room temperature in the order given (see Note 4):

x µL	Nuclease-Free Water, Sterile
1 µg	linearised template DNA with appropriate promoter (see Note 3)
2 µL	AmpliScribe T7 10X Reaction Buffer
1.5 µL	100 mM ATP
1.5 µL	100 mM CTP
1.5 µL	100 mM GTP
1.5 µL	100 mM UTP
2 µL	100 mM DTT
0.5 µL	RiboGuard RNase Inhibitor
2 µL	AmpliScribe T7 RNA Polymerase
20 µL	Total reaction volume

6. Scaled-up AmpliScribe high yield transcription reaction

An AmpliScribe reaction can be scaled up to produce milligram amounts of RNA. To maximise RNA yield, all reaction components, including the template DNA, should be scaled up proportionally.

Important! Bring all reaction components, except the AmpliScribe T7 RNA Polymerase, to room temperature, then:

1. Combine and mix the appropriate volume of each reaction component, except the AmpliScribe T7 RNA Polymerase, in the order given in the Standard AmpliScribe High Yield Transcription Reaction procedure.
2. Add the appropriate volume of the AmpliScribe T7 RNA Polymerase and mix.
3. Incubate the reaction for 2 hours at 37 °C.
4. Optional: Treat the sample with RNase-Free DNase I to remove template DNA and purify the RNA (see Section 8).

7. DNase I treatment

If removal of the DNA template is desired, digest the sample with RNase-free DNase I.

1. Add 1 µL (1 MBU) of RNase-Free DNase I to the standard 20 µL AmpliScribe Transcription reaction and incubate for 15 minutes at 37 °C.
2. Extract with TE-saturated phenol/chloroform, followed by chloroform. Ethanol- precipitate the RNA or precipitate the RNA using ammonium acetate as described in Purification of the RNA. RNA may be cleaned up also using Zymo RNAClean and Concentrator or Qiagen RNEasy spin columns, or by using ammonium acetate.

8. Purification of the RNA

RNA transcripts >100 bases can be purified by ammonium acetate precipitation without ethanol.
This method selectively precipitates RNA while leaving much (but not all) of the DNA, protein, and unincorporated NTPs in the supernatant.

1. Add one volume of 5 M ammonium acetate (20 µL for the standard AmpliScribe reaction).
2. Incubate on ice for 10-15 minutes.
3. Centrifuge at high speed (e.g., ≥10,000 x g) for 10-15 minutes at room temperature or 4 °C.
4. Wash the pellet in 70% ethanol.
5. Store the RNA at -20 °C or -70 °C as a dry pellet or resuspended in Nuclease-Free Water, Sterile; TE; or other suitable buffer.

For RNA transcripts <100 bases, remove the unincorporated NTPs by chromatography followed by ethanol precipitation.

1. Remove unincorporated NTPs by spin-column chromatography. For commercially available columns, follow the manufacturer's instructions for this step.
2. Add sodium acetate to a final concentration of 0.3 M, followed by 2.5 volumes of ethanol.

3. Incubate at -20 °C for 30 minutes and collect the RNA by centrifugation.
4. Remove the supernatant carefully with a pipette and gently rinse the pellet with 70% ethanol.
5. Store the RNA at -20 °C or -70 °C as a dry pellet or re-suspended in Nuclease-Free Water, Sterile; TE; or other suitable buffer.

9. Preparing non-radioactively-labeled RNA using the AmpliScribe High Yield Transcription Kits

Fluorescent-labeled RNA can be prepared by either direct incorporation of fluorescent-labeled NTPs in a T7 AmpliScribe reaction or by post-transcriptional labeling of the RNA with fluorescent compounds using post transcriptional labeling products from third party vendors. The protocol provided here describes direct incorporation of Cy®5-UTP into RNA in an AmpliScribe reaction. For a detailed protocol for incorporating fluorescein-12-UTP and fluorescein-12-CTP or for post-transcriptional labeling with Cy3, see DeLong, E.F. *et al.*[1] Biotin-labeled[2] and digoxigenin-labeled[3] RNA can also be prepared by the modifying the standard AmpliScribe reaction.

1. *Important!* Combine the following reaction components at room temperature in the order given (see Note 4):

x	µL Nuclease-Free Water, Sterile
1	µg linearised template DNA with T7 promoter
2	µL AmpliScribe T7 10X Reaction Buffer
1.5	µL 100 mM ATP
1.5	µL 100 mM CTP
1.5	µL 100 mM GTP
1	µL 100 mM UTP
6	µL 4 mM Cy5-UTP
2	µL 100 mM DTT
0.5	µL RiboGuard RNase Inhibitor
2	µL AmpliScribe T7 RNA Polymerase
20	µL Total reaction volume

2. Incubate at 37 °C for 2 hours.
3. Optional: Treat the sample with RNase-Free DNase I to remove template DNA and purify the fluorescent-labeled RNA, or as described in the product literature for Cy5-UTP.

10. Analysis of the RNA transcript

Use a denaturing agarose gel[4] of appropriate concentration for the RNA transcription product.

Denaturing gels allow *in vitro* transcripts to separate on the basis of their length rather than based on their length plus secondary structure. Denaturing conditions for electrophoresis will remove any secondary structures from the RNA and allow the RNA to migrate in a tight band rather than a smear which can occur with native gels.

11. References

1. DeLong, E.F. *et al.,* (1999) *Appl. and Environ. Microbiol.,* **56,** 5554.
2. Hoffman, L.M. and Johnson, M.G. (1994) *BioTechniques* **17,** 372.
3. Kaplan, E.D. *et al.,* (1996) *Epicentre Forum,* **3** (2), 1.
4. Molecular Cloning - A Laboratory Manual, Third Edition, 2001. CSHL Press. pp 7.27 – 7.34. J. Sambrook and D. Russell.

12. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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