

Consistent Production of the Highest Possible RNA Yields with AmpliScribe™ High Yield Transcription Kits

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Introduction

In vitro transcription of DNA templates by a phage RNA polymerase is a commonly used, efficient method for generating specific RNA transcripts. A phage RNA polymerase directs the synthesis of RNA from a cloned, linearized DNA template under the control of a specific phage promoter. RNA produced by *in vitro* transcription is used in a variety of applications including *in vitro* translation, ribozyme synthesis¹, splicing or processing studies, structural determinations, and the creation of non-radioactive RNA probes.²⁻⁴ RNA transcripts produced by transcription reactions are also often microinjected into cells⁵ for anti-sense experiments, or used for cellular localization of RNA.⁴

EPICENTRE'S AmpliScribe™ T7, T3, and SP6 High Yield Transcription Kits have been specially formulated to utilize high concentrations of NTPs that are inhibitory to other kits and conventional, *in vitro* transcription systems. The result is the highest possible yield of RNA transcripts, which can range in size from 25 bases to several kilobases.⁶ Using AmpliScribe's T7 RNA transcription reaction can generate up to 150 µg of high quality, full-length RNA transcript (1.4 Kb) per µg of template DNA. Thus, up to 90% of the NTPs present are incorporated into RNA product.

Here the performance of the AmpliScribe T7 Transcription Kit was compared with a conventional T7 RNA polymerase transcription reaction⁷ as well as similar transcription kits from two leading suppliers. Comparative analyses of *in vitro* transcription reaction products were based on both the yield and the integrity of the RNA produced.

Materials and Methods

Preparation of DNA Templates

Linear DNA templates were generated by restriction enzyme digestion of plasmid DNA. The digested DNA was treated with 200 µg/ml Proteinase K and 0.5% SDS for 30 minutes at 50°C to minimize nuclease contamination. Plasmids were then purified by phenol/chloroform extraction, ethanol precipitated, and resuspended in TE buffer. The AmpliScribe linear control DNA was used as supplied in the kit.

Transcription Reactions

AmpliScribe T7 transcription reactions were performed according to the protocol provided in the kit. Briefly, 20 µl reactions contained 1X Reaction Buffer, 10 mM DTT, 7.5 mM each NTP, 1 µg linearized DNA template, and 2 µl

AmpliScribe T7 Enzyme Solution. Reactions were incubated at 37°C for 2 hours, unless otherwise indicated.

The transcription reactions performed with other manufacturers' kits were performed according to the protocols provided. Each 20 µl reaction contained 1 µg linearized DNA template and was incubated at 37°C for 2 hours, unless otherwise indicated. The conventional transcription reaction contained 10 U of T7 RNA Polymerase, 0.5 mM each NTP, in 1X transcription buffer with 10 mM DTT.⁷

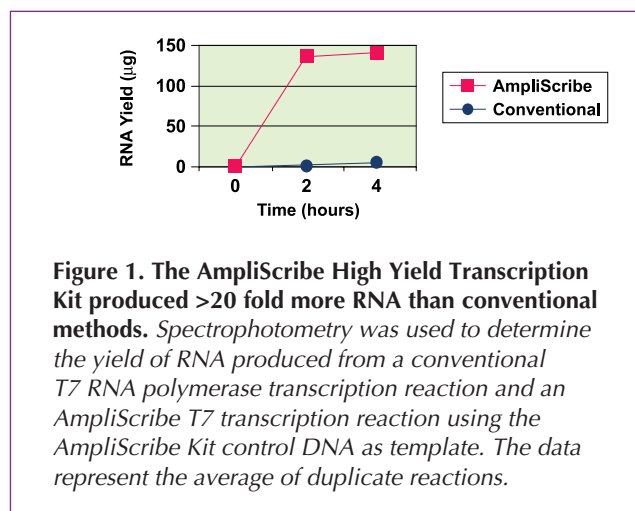
RNA Quantitation and Integrity Analysis

Transcription reactions were stopped by the addition of an equal volume of cold 5 M NH₄OAc. The samples were chilled on ice for 10 minutes and the RNA was pelleted in a microcentrifuge for 10 minutes at full speed. The RNA samples were resuspended in TE, quantitated by spectrophotometry, and analyzed for integrity by electrophoresis on native agarose gels using standard methods.

Results and Discussion

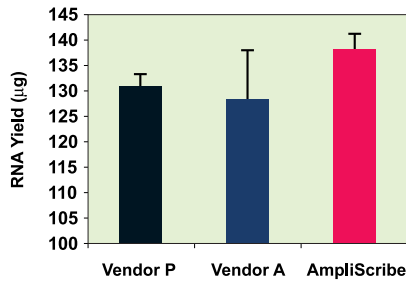
The AmpliScribe High Yield Transcription Kits produced >20 fold more RNA than conventional methods

Timed transcription reactions were performed using the AmpliScribe T7 High Yield Transcription Kit and a conventional T7 RNA polymerase method⁷ in order to produce a 1.4 Kb transcript. Reactions were incubated for 2 or 4 hours at 37°C and stopped by ammonium acetate precipitation. The RNA yields produced, as determined by spectrophotometry, are depicted in Figure 1.

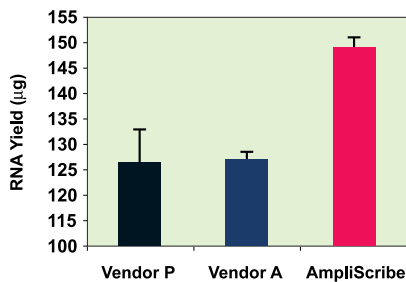


continued

A. RNA Yield with 2.6 Kb Template



B. RNA Yield with 1.8 Kb Template



C. RNA Yield with 63 Base Template

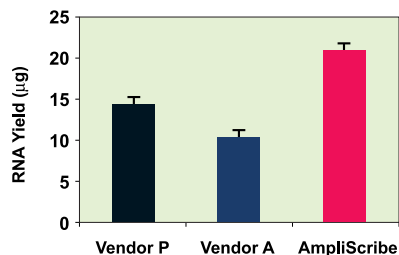


Figure 2. AmpliScribe High Yield Transcription Kits consistently produce the highest possible RNA yields. Spectrophotometry was used to determine the yield of RNA produced by 2-hour transcription reactions with the AmpliScribe T7 Kit and the kits of two competitors. The data represent the average of duplicate reactions with standard errors. The transcripts produced were A) 2.6 kb, B) 1.8 kb, and C) 63 bases long from different DNA templates.

The AmpliScribe T7 transcription reaction produced > 20 fold more full-length RNA transcript than the conventional reaction.

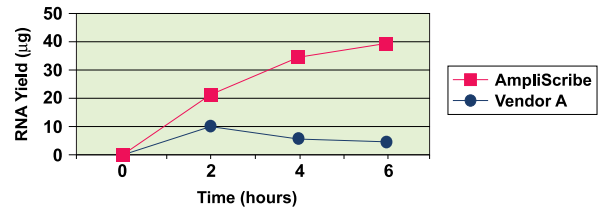


Figure 3. The AmpliScribe T7 High Yield Transcription Kit produces higher yields of short (<300 base) RNA than a transcription kit specifically for short templates. The yield of a 63 base RNA was compared at 2, 4, and 6 hour time points. The average yield of duplicate reactions is shown.

AmpliScribe High Yield Transcription Kits consistently produce the highest possible RNA yields

Comparisons were performed with the AmpliScribe T7 High Yield Transcription Kit and high yield transcription kits from two other leading suppliers. Two-hour transcription reactions were performed on three linear DNA templates varying from 63 bases to 2.6 kilobases in length, following the manufacturers' recommended protocols. Figure 2 shows that the AmpliScribe Kit consistently produced more transcripts than either of the other high yield transcription kits (e.g., 150 µg of the 1.8 Kb RNA in 2 hours versus less than 128 µg for vendors A and P).

The AmpliScribe T7 High Yield Transcription Kit produces higher yields of short (<300 bases) RNA

Producing large quantities of a short transcript requires more transcription initiation events than with a standard (e.g., 1 Kb) template. Figure 2C shows that the amount of a short (63 base) transcript produced with the AmpliScribe T7 High Yield Transcription Kit exceeded the amount produced with high yield kits from vendors A and P. Vendor A also markets a T7 transcription kit specifically designed for producing short (<300 base) RNA. In order to determine how the AmpliScribe T7 High Yield Transcription Kit compared with this suppliers "short transcription" kit, comparative reactions were performed.

As shown in Figure 3, the AmpliScribe Kit clearly produced more of the 63 base transcript than the kit designed for short templates. The standard two-hour AmpliScribe transcription reaction produced approximately twice as much RNA as the other system. Lengthening the reaction incubation to 6 hours increased the yield to as much as 4 times the amount using vendor A's specialized kit. Note that although the number of micrograms of short RNA produced is small compared to the yield of > 1 Kb RNA, the number of moles of short RNA produced is greater than for a >1 Kb RNA. Some RNA degradation was also detected with vendor A's kit after 6 hours, while the AmpliScribe Kit continued to accumulate full-length RNA transcripts.

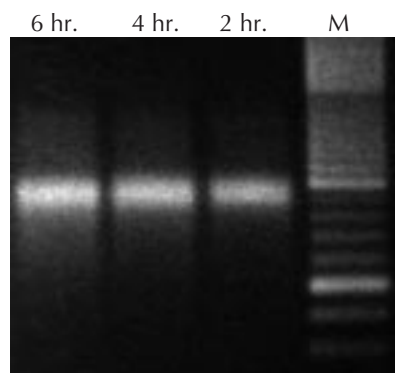


Figure 4. RNA with excellent integrity is produced with the AmpliScribe T7 High Yield Transcription Kit. Agarose gel electrophoresis of the 1.4 kb transcript produced from 2, 4, and 6 hour transcription reactions with the AmpliScribe T7 Kit. M, DNA ladder.

Excellent RNA Integrity with the AmpliScribe T7 High Yield Transcription Kit

Ideally *in vitro* transcription reactions should yield full-length, intact RNA transcripts. In order to compare the integrity of RNA transcripts produced, agarose gel electrophoretic analyses were performed on transcription products made using the AmpliScribe T7 Kit and high yield kits from vendors A and P. All of the transcripts produced by the kits tested produced primarily high-quality, full-length, 1.8 Kb RNA transcripts (data not shown). In the time course experiment shown in Figure 4, the integrity of the 1.4 Kb transcript produced by the AmpliScribe T7 Kit is shown after 2, 4 and 6 hour reactions. All time points yielded high quality, full-length RNA.

Summary

The AmpliScribe T7 High Yield Transcription Kit consistently produces the highest possible RNA yields of any *in vitro* transcription system available. The AmpliScribe Kit efficiently uses a broad range of DNA templates, to synthesize reproducibly large amounts of full-length RNA transcript.

References

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AmpliScribe™ High Yield Transcription Kits

AmpliScribe™ T7 High Yield Transcription Kit

AS2607-F72	25 Reactions
AS3107-F72	50 Reactions

AmpliScribe™ T3 High Yield Transcription Kit

AS2603-F72	25 Reactions
AS3103-F72	50 Reactions

AmpliScribe™ SP6 High Yield Transcription Kit

AS2606-F72	25 Reactions
AS3106-F72	50 Reactions

Each kit includes RNA Polymerase (with added RNase inhibitor), 10X Reaction Buffer, 100 mM each NTP, RNase-free DNase I, DTT, and Control DNA template.

AmpliScribe Kits with Cap Analog (Methylated)

25 U Cap Analog

T7 Polymerase Kit

AS2607C2-F72	25 Reactions
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SP6 Polymerase Kit

AS2606C2-F72	25 Reactions
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T3 Polymerase Kit

AS2603C2-F72	25 Reactions
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50 U Cap Analog

T7 Polymerase Kit

AS2607C5-F72	25 Reactions
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SP6 Polymerase Kit

AS2606C5-F72	25 Reactions
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T3 Polymerase Kit

AS2603C5-F72	25 Reactions
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