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

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Introduction

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. Yields of up to 150 µg of full length RNA per µg of DNA template in a standard 20 µl reaction can be consistently realized.

Here we compare the performance of the AmpliScribe T7 High Yield Transcription Kit with *in vitro* transcription kits from two other suppliers and with a conventional T7 RNA Polymerase transcription reaction. Comparative analyses of *in vitro* transcription reaction products were based on both the yield and the integrity of the RNA produced.

Methods

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 50ºC 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.

Standard 20 µl *in vitro* transcription reactions were performed according to the protocol provided in the kits tested. Each 20 µl reaction contained 1 µg of linearized DNA template and was incubated at 37ºC 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 as described in reference 1.

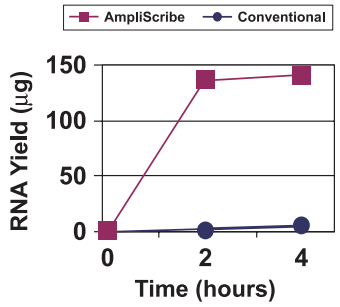
Transcription reactions were stopped and RNA transcripts were purified from unincorporated NTPs by the addition of an equal volume of cold 5 M NH_4 OAc. The samples were chilled on ice for 10 minutes and the RNA was pelleted in a microcentrifuge for 10 minutes at full speed. The RNA samples were resuspended in TE, quantitated by spectrophotometry, and analyzed for integrity by electrophoresis on native agarose gels.

Results

AmpliScribe Kits produce >20 fold more RNA than conventional methods

Transcription reactions were performed for 2 hours and 4 hours using the AmpliScribe T7 High Yield Transcription Kit and a conventional T7 RNA Polymerase method.[1] Figure 1 shows that the AmpliScribe T7 Transcription Kit produced > 20 fold more full length 1.4 Kb RNA transcript than the conventional reaction.

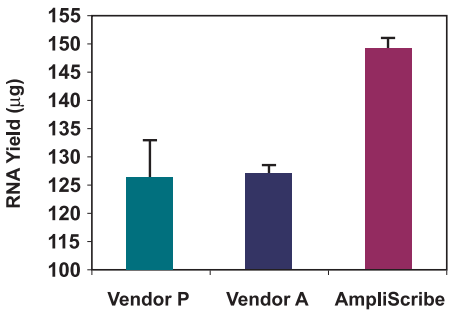
Figure 1. The AmpliScribe™ T7 High Yield Transcription Kit consistently produced >20 fold more of a 1.4 Kb RNA than a conventional T7 RNA Polymerase reaction.



AmpliScribe T7 High Yield Transcription Kits consistently produce the highest yield of long RNA

Yields of a 1.8 Kb RNA transcript produced using the AmpliScribe T7 High Yield Transcription Kit and transcription kits from two other suppliers were compared. The AmpliScribe High Yield Kit consistently produced more of the 1.8 Kb RNA in a standard 20 µl, 2-hour reaction than the other kits (Figure 2).

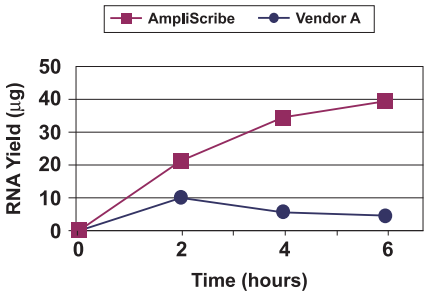
Figure 2. AmpliScribe™ T7 High Yield Transcription Kit consistently produced the highest yield of a 1.8 Kb RNA. Up to 150 µg of RNA was produced using the AmpliScribe™ T7 High Yield Kit in a standard 20 µl, 2-hour reaction.



The AmpliScribe Kit produces the highest yields of short (<300 bases) RNA

Producing large quantities of a short transcript requires more transcription initiation events than with production of a larger (e.g., 1 Kb) transcript. The AmpliScribe T7 High Yield Transcription Kit was compared to Vendor A's "short transcription" kit specifically designed for producing short RNA. As shown in Figure 3, the AmpliScribe Kit produced twice as much of a 63 base RNA transcript in a standard 2-hour reaction than the competitor's kit designed for producing short transcripts. Lengthening the reaction incubation to 6 hours increased

Figure 3. The AmpliScribe™ T7 High Yield Transcription Kit produces higher yields of short (<300 base) RNA than a competitor's kit specifically designed for short templates. The yield of a 63 base RNA was compared at 2, 4, and 6 hour time points.



the yield from the AmpliScribe Kit to as much as 4 times the amount produced using Vendor A's specialized kit. Some RNA degradation was also detected with Vendor A's kit after a 6 hour reaction, while the AmpliScribe Kit continued to accumulate full-length RNA transcripts. Note that although the number of micrograms of the 63 base RNA produced is small compared to the yield of the 1.8 Kb RNA, the number of moles of 63-base RNA produced is greater.

Excellent RNA integrity with the AmpliScribe T7 Kit

The AmpliScribe RNA Polymerases contain an added RNase inhibitor. We analyzed the integrity of RNA transcripts produced using the AmpliScribe T7 Kit by agarose gel electrophoresis. Figure 4 presents agarose gel analysis of a 1.4 Kb RNA transcript produced by the

AmpliScribe T7 Kit 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 RNA yields of any *in vitro* transcription system available using a broad range of DNA templates.

References

1. Melton, D. *et al.* (1984) Nucl. Acids Res.**12**, 7035.

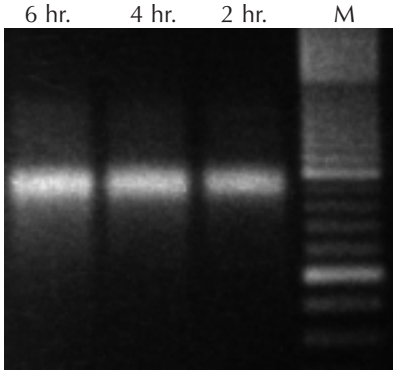


Figure 4. RNA with excellent integrity is produced with the AmpliScribe™ T7 High Yield Transcription Kit. Agarose gel electrophoresis of the 1.4 Kb transcript produced from 2, 4, and 6 hour transcription reactions with the AmpliScribe T7 Kit. M, DNA ladder.

AmpliScribe™ T7 High Yield Transcription Kit

AS2607	25 Reactions
AS3107	50 Reactions

AmpliScribe™ T3 High Yield Transcription Kit

AS2603	25 Reactions
AS3103	50 Reactions

AmpliScribe™ SP6 High Yield Transcription Kit

AS2606	25 Reactions
AS3106	50 Reactions

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

Quantity discount on AmpliScribe Kits is available. Please inquire.

Highest Yield of 5'-Capped RNA from an *In Vitro* Transcription Reaction with AmpliCap™ High Yield Message Maker Kits

EPICENTRE's AmpliCap™ T7, T3 and SP6 High Yield Message Maker Kits are specially formulated to produce the highest yield of 5′-capped RNA from an *in vitro* transcription reaction. A Cap/NTP PreMix containing optimal concentrations of $m^7G[5']ppp[5']G$ Cap analog and NTPs, is provided to maximize capping efficiency and RNA yield.

The new AmpliCap™ T7, SP6 and T3 High Yield Message Maker Kits feature:

- Capped RNA yields of up to 45 µg from the T7 and T3 kits and up to 35 µg using the SP6 kit.
- Up to 80% of the RNA is capped using all three kits.
- An optimized $m^7G[5']ppp[5']G$ Cap/NTP PreMix solution is provided for ease of use and highest yields of capped RNA transcripts.
- A separate vial of GTP for efficient production of long, 5′-capped RNA.

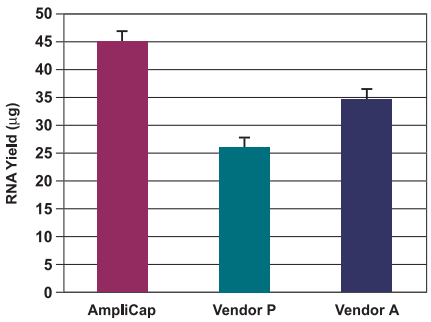


Figure. AmpliCap™ T7 High Yield Message Maker Kit consistently produces the highest yield of 5′-capped RNA transcript. A standard 20 µl reaction produces more 5′-capped transcript than the competitor's kits.

AmpliCap™ High Yield Message Maker Kits

T7

AC0707	25 Reactions

T3

AC0703	25 Reactions

SP6

AC0706	25 Reactions

Each kit contains the respective RNA Polymerase (including RNase inhibitor), Cap/NTP PreMix, 20 mM GTP 10X AmpliCap™ Transcription Buffer, 100 mM DTT, RNase-free DNase I, Control template DNA, RNase-free water