

Transcribe More RNA in 30 Minutes Than Other *In Vitro* Transcription Kits Produce in 2 Hours

Using the AmpliScribe™ T7-Flash™ Transcription Kit

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Introduction

The ability to transcribe large amounts of RNA, *in vitro*, is critical for the success of many RNA-dependent techniques, including gene expression, RNA processing, ribozymes, *in situ* hybridization, and microinjection studies. The AmpliScribe™ T7-Flash™ Transcription Kit, EPICENTRE's new T7 *in vitro* transcription kit, is specially formulated to produce the highest yield of RNA from an *in vitro* transcription reaction in the shortest amount of time. Here we report on the different parameters of an AmpliScribe T7-Flash reaction that affect the yield of both long and short RNA transcripts.

Methods

AmpliScribe T7-Flash Transcription reactions were performed as described in the product literature. Briefly, the standard 20- μ l reaction contained 1 μ g of linear, double-stranded DNA template and was performed at 37°C for 30 minutes, unless otherwise indicated. After the reaction, transcripts larger than 1 kb were purified by ammonium acetate precipitation. Ammonium acetate selectively precipitates the large RNA while leaving the DNA and unincorporated nucleotides in solution. The purified RNA was resuspended in RNase-Free Water. For transcripts less than 1 kb in size, the completed reaction was digested with DNase I and the RNA was purified by column chromatography. RNA yield was quantified by absorbance at 260 nm. The integrity of the RNA was checked by electrophoresis on a denaturing, 1% agarose-formaldehyde gel.

Results

An AmpliScribe T7-Flash reaction is complete in 30 minutes and produces more RNA than other kits do in 2 hours

The AmpliScribe T7-Flash Transcription Kit has been formulated to transcribe large amounts of RNA in 30 minutes. In comparison, other commercial, high yield T7 *in vitro* transcription kits require at least 2 hours to maximize RNA production. As shown in Figure 1, the AmpliScribe T7-Flash reaction was complete in 30 minutes and produced about 3-fold more of a 1.4-kb RNA transcript than the competitive kit produced in the

same amount of time, from the same template. Even after 2 hours, when the competitive kit's reaction was finally complete it had produced less RNA than the AmpliScribe T7-Flash reaction had in 30 minutes.

The reaction kinetics for transcribing short (<1 kb) RNAs are discussed below.

In a related series of experiments, the AmpliScribe T7-Flash reaction time was reduced to as little as 15 minutes by increasing the amount of DNA template in the reaction (data not shown).

AmpliScribe T7-Flash reactions produce exceptionally high yields of RNA

AmpliScribe T7-Flash reactions consistently produced 160 to 180 μ g (8-9 mg/ml) of a 1.4-kb RNA from 1 μ g of the control DNA template included with the kit. Yields of up to 200 μ g of RNA were produced from 1 μ g of other DNA templates that were tested (Table 1). The RNA produced was intact and full-length (Figure 2). AmpliScribe T7-Flash reactions can also be scaled-up to produce milligram amounts of RNA (data not shown).

RNA yield from limiting amounts of DNA template

For some *in vitro* transcription experiments, the amount of DNA template may

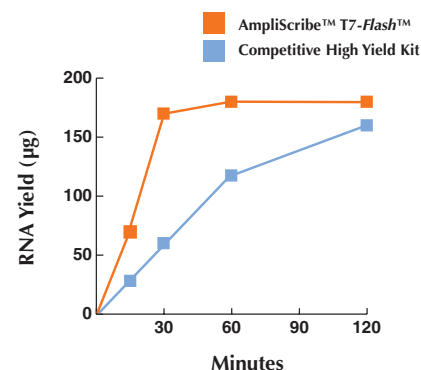


Figure 1. An AmpliScribe™ T7-Flash™ transcription reaction is complete in 30 minutes and produces more RNA than a competitive, high yield transcription reaction produces in 2 hours. In a comparison, 1 μ g of a linear DNA template, producing a 1.4-kb RNA transcript, was transcribed in an AmpliScribe T7-Flash reaction and in an *in vitro* transcription reaction from a competitive kit. RNA samples were purified and quantified as described in the article.

be limiting. Therefore, we investigated the yield of RNA from AmpliScribe T7-Flash reactions containing less than 1 μ g of the control DNA template (Figure 3). High yields of RNA were obtained in 30 minutes from as little as 100 ng of template. Increasing the reaction time or increasing the reaction temperature from 37°C to 42°C improved the yield of RNA from reactions containing less than 1 μ g

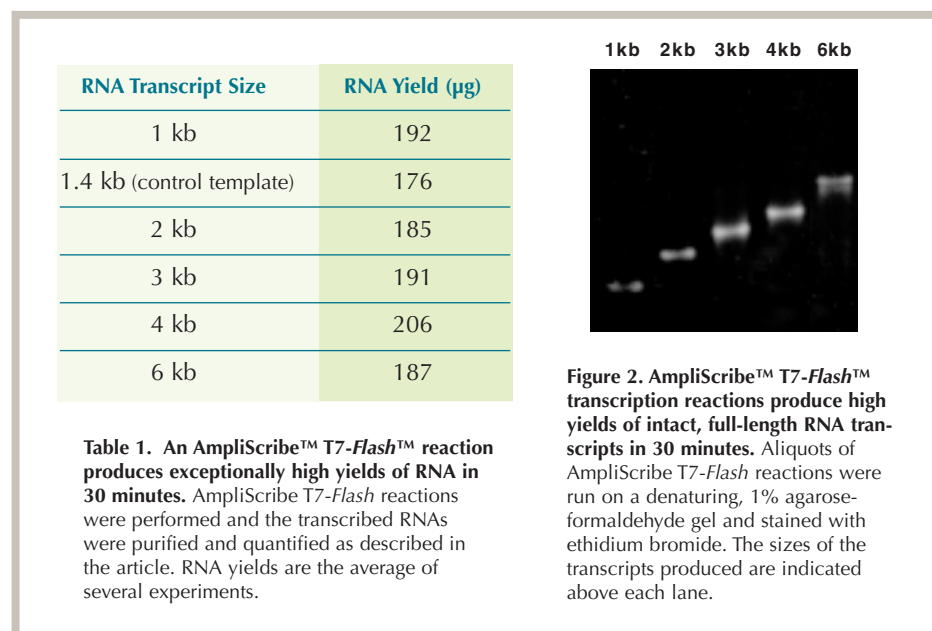
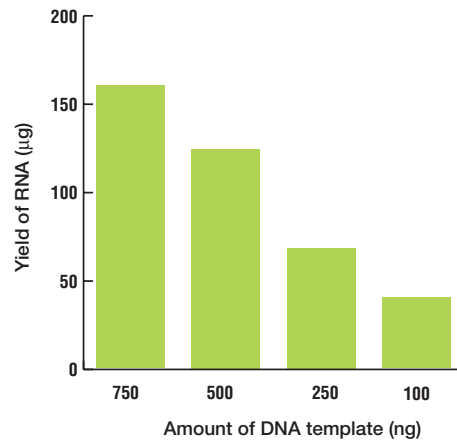


Figure 2. AmpliScribe™ T7-Flash™ transcription reactions produce high yields of intact, full-length RNA transcripts in 30 minutes. Aliquots of AmpliScribe T7-Flash reactions were run on a denaturing, 1% agarose-formaldehyde gel and stained with ethidium bromide. The sizes of the transcripts produced are indicated above each lane.

Table 1. An AmpliScribe™ T7-Flash™ reaction produces exceptionally high yields of RNA in 30 minutes. AmpliScribe T7-Flash reactions were performed and the transcribed RNAs were purified and quantified as described in the article. RNA yields are the average of several experiments.

Figure 3. High yields of RNA can be obtained from limiting amounts of DNA template in an AmpliScribe™ T7-Flash™ transcription reaction.

Decreasing amounts of a linear DNA template, producing a 1.4-kb RNA transcript, were transcribed for 30 minutes in AmpliScribe T7-Flash reactions. RNA samples were purified and quantified as described in the article. RNA yield from limiting amounts of template can be increased by increasing the reaction time or increasing the reaction temperature to 42°C.



of DNA template. For example, increasing the reaction time to 2 hours produces the maximum yield (>160 µg) of RNA from 250 ng of template.

Transcription of short RNA transcripts using the AmpliScribe T7-Flash Transcription Kit

Efficient transcription of short RNAs (e.g. short hairpin RNA; see page 1) is important for many RNA interference (RNAi) studies. A variety of templates producing RNA transcripts from 26 to 335 bases were transcribed using the AmpliScribe T7-Flash Kit and another commercial, high yield transcription kit. In all cases tested, the AmpliScribe T7-Flash reaction produced more RNA than the other transcription kit. Though the microgram amounts of short transcripts produced seems low, the molar amount of short RNA produced was greater than the molar amount of long (>1 kb) transcripts generated (Table 2). Since many RNAi studies utilize on the order of 10 pmoles of RNA, a single AmpliScribe T7-Flash reaction produces enough short RNA for more than 100 RNAi experiments. The yield of short RNAs can be increased by:

Increasing reaction times.

Increasing the amount of DNA template.

Increasing the reaction temperature to 42°C.

For example, when transcribing a 26-bp template, maximum yields are obtained after a 4-hour incubation at 37°C. The reaction time can be reduced to 2 hours if done at 42°C, or in as little as 1 hour if done at 42°C using 3 µg of template.

Table 2. AmpliScribe™ T7-Flash™ reactions produce high yields of short RNA transcripts in a 30-minute reaction. Extending the reaction time, increasing the amount of template in the reaction, or increasing the reaction temperature to 42°C will increase yields of short RNA transcripts.

RNA Transcript Size	RNA Yield (µg)	RNA Yield (pmoles)
26 bases	12	1319
47 bases	24	1459
96 bases	36	1071
335 bases	76	648
1.4 kb	176	359
6 kb	187	89

Conclusion

The AmpliScribe T7-Flash Transcription Kit is ideal for *in vitro* transcription of RNA because it provides the highest yields of RNA in the shortest reaction time. Transcription of long RNA transcripts was complete in 30 minutes compared to the 2-hour reaction required for other transcription systems. RNA yields as high as 200 µg were observed with some templates. Short RNA transcripts are also efficiently produced by the AmpliScribe T7-Flash Transcription Kit. A single 20-µl reaction produces enough picomoles of short RNA for at least 100 RNAi experiments (see article on page 1).

www.epicentre.com/t7-flash.asp

AmpliScribe™ T7-Flash™ Transcription Kit

ASF3257 25 Reactions

ASF3507 50 Reactions

Contents:

AmpliScribe™ T7-Flash™ Enzyme Solution (with RNase inhibitor), AmpliScribe™ T7-Flash™ 10X Reaction Buffer, 100mM ATP, CTP, GTP, UTP Solutions, RNase-Free DNase I, DTT, RNase-Free Water, and Control DNA Template (linearized).

T7 In Vitro Transcription In A FLASH!

New! AmpliScribe™ T7-Flash™ Transcription Kit

- 30 Minute T7 In Vitro Transcription Reactions
- Highest yields of RNA