

Generate High Yields of Biotin-Labeled RNA Using the AmpliScribe™ T7-Flash™ or the AmpliScribe™ T3-Flash™ Transcription Kits

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EPICENTRE's new AmpliScribe™ T7-Flash™ and AmpliScribe™ T3-Flash™ Transcription Kits produce the highest yield of RNA transcripts from an *in vitro* transcription reaction in the shortest reaction time. Previously, we demonstrated that an AmpliScribe T7-Flash reaction produces 160 to 180 µg (8 to 9 mg/ml) of RNA in a 30 minute reaction^{1,2}...more RNA than other kits produce in 2 hours. Subsequently, we have shown that an AmpliScribe T7-Flash reaction can be scaled up to produce milligram quantities of RNA in 30 minutes³ and that AmpliScribe T7-Flash reactions produce exceptionally high yields of short (<500 bases) RNA transcripts.⁴

Here we report reaction conditions that produce high yields of biotinylated-RNA using the AmpliScribe T7-Flash or AmpliScribe T3-Flash Kits.

1. Assemble the reaction components at room temperature.

- X µl RNase-Free water, for a final reaction volume of 20 µl
- 1.0 µg linear template DNA with a T7 or T3 promoter
- 2.0 µl AmpliScribe™ T7-Flash™ or AmpliScribe™ T3-Flash™ 10X Reaction Buffer
- 1.8 µl 100 mM ATP
- 1.8 µl 100 mM CTP
- 1.8 µl 100 mM GTP
- 1.3 µl 100 mM UTP
- 5.0 µl 10 mM Biotin-16-UTP[†] (Roche)
- 2.0 µl 100 mM DTT
- 2.0 µl AmpliScribe™ T7-Flash™ or AmpliScribe™ T3-Flash™ Enzyme Solution

20 µl Total reaction volume.

2. Incubate the reaction at 37°C for 30 minutes.
3. Add 1µl of RNase-Free DNase I (included in the kits) and incubate at 37°C for 15 minutes to remove the DNA template.
4. Purify the biotin-labeled RNA as described in the AmpliScribe T7-Flash and AmpliScribe T3-Flash product literature.

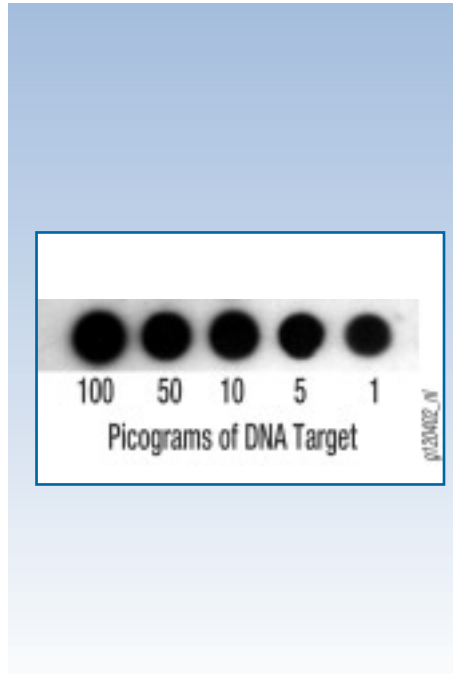


Figure 1. Dot-blot analysis of biotinylated-RNA produced from an AmpliScribe™ T7-Flash™ reaction. Serial dilutions of unlabeled, control-plasmid template DNA, supplied in the AmpliScribe T7-Flash Kit, were spotted onto a Nytran® SPC membrane (Schleicher and Schuell) using a vacuum manifold. The membrane was probed with 200 ng of biotinylated-RNA, about 0.1% of the biotinylated-RNA produced by the reaction procedure described in the text. Hybridized RNA was detected by chemiluminescence using a streptavidin-alkaline phosphatase conjugate and CDP-Star® substrate with the Southern-Star™ System (ABI/Tropix), according to the manufacturer's protocol. The biotinylated-RNA detected 1 pg of target DNA with a 15 second exposure.

Results

AmpliScribe T7-Flash or T3-Flash transcription reactions are easily modified to incorporate biotin-rNTP nucleotides. The 30-minute transcription reaction described above yields ≥160 µg (>8 mg/ml) of biotinylated-RNA using the 1.4 kb control DNA template provided in the kits. As shown in Figure 1, 200 ng of the resulting biotinylated-RNA detected as little as 1 pg of target DNA in a dot-blot assay. An AmpliScribe T7-Flash or AmpliScribe T3-Flash transcription reaction produces enough biotinylated RNA to do more than 800 comparable experiments.

† Biotin-nucleotides are not supplied with the AmpliScribe T7-Flash or AmpliScribe T3-Flash Kits. Biotin-rNTPs, other than UTP, may be used, but a final concentration of 2.5 mM biotin-rNTP and 6.5 mM unlabeled rNTP should be maintained in the transcription reaction.

References

1. Meis, R. and Pease, J. (2003) *EPICENTRE Forum* **10**(2) 6.
2. Meis, R. *et al.* (2003) *Bioscience Technology*, **28**(9) 8.
3. *EPICENTRE Forum* **10**(3) 8.
4. Meis, R. (2004) *EPICENTRE Forum* **11**(1) 7.

www.epicentre.com/flash.asp

AmpliScribe™ T7-Flash™ Transcription Kit

ASF3057-F12	5 Reactions	\$ 50
ASF3257	25 Reactions	
ASF3507	50 Reactions	

AmpliScribe™ T3-Flash™ Transcription Kit

ASF03725	25 Reactions
ASF03750	50 Reactions

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* CDP-Star is a registered trademark and Southern-Star is a trademark of Applera Corp., Norwalk, CT.