



Ask Frank

By Fred and Hank



AmpliScribe™ T7-Flash™ and DuraScribe® *In Vitro* Transcription Kits

Q: What is the longest *in vitro* transcript readily made with an AmpliScribe™ T7-Flash™ Transcription Kit?

A: While we have tested and readily produced 9-kb transcripts at EPICENTRE, our customers report high-quality transcripts greater than 11 kb.

Q: I'm using less than the recommended 1 µg of DNA template in an AmpliScribe T7-Flash reaction. How can I improve my RNA yield?

A: With lower concentrations of DNA template, you can increase RNA yields by increasing the reaction time from the recommended 30 minutes up to 2-4 hours, depending on the amount of template used. Increasing the reaction temperature from 37°C to 42°C also improves the yield.

Q: Why should I set up the AmpliScribe T7-Flash reactions at room temperature? Won't that affect the RNA polymerase enzyme activity?

A: The components of the reagents in a standard 20-µl reaction are very close to their solubility limits. If you set up the reaction on ice, some reaction components (such as the buffer and the nucleotides) will precipitate. After components precipitate, warming the reaction tube only partially resolubilizes the reagents. The AmpliScribe T7-Flash Enzyme Solution is added last, so it can be kept on ice until needed.

Q: How clean does the template DNA need to be for *in vitro* transcription?

A: Very clean template DNA ensures the best performance of an *in vitro* transcription reaction. If using a PCR product for the template, purify the desired product from

the reaction to remove any remaining primers, primer-dimers and residual dNTPs. If you are using a plasmid for the template, in order to transcribe the desired RNA product, completely linearize the plasmid, leaving a blunt or 5'-overhanging end. Uncut plasmid serves as excellent template, but the RNA polymerase will transcribe past the desired transcription stop point and can continue around the plasmid several times before the reaction finally stops, creating a RNA that is far longer than desired and includes undesirable vector sequences.

Q: What is the shortest *in vitro* transcript readily made with an AmpliScribe T7-Flash Transcription Kit?

A: We have produced 26-base RNA transcripts with an AmpliScribe T7-Flash Transcription Kit.

Q: Can I make nonradioactive, labeled RNA using an AmpliScribe T7-Flash Kit (with labeled nucleotides or by end-labeling)?

A: Yes, you can directly incorporate derivatized nucleotides (with moieties like Cy5, biotin, or digoxigenin) into the transcripts or you can perform post-transcriptional labeling of purified RNA transcripts at the 5' or 3' ends. Please contact us for specific protocols.

Q: What advantages does an AmpliScribe T7-Flash Transcription Kit have over the standard AmpliScribe High-Yield Transcription Kit?

A: The two main advantages of the AmpliScribe T7-Flash™ Kit are: i) improved RNA yields, even better than the excellent results obtained with the AmpliScribe High-Yield Transcription Kits; and ii) a fast, 30-minute procedure.

Q: Can I make radiolabeled probes with any of the AmpliScribe High-Yield or T7-Flash transcription kits?

A: Yes. While rarely done any more, radioactive probes may be made using post-transcriptional labeling. Generating radioactive RNA during the *in vitro* transcription reaction requires a lot of radioactive nucleotide, due to the high concentrations of radioactive NTPs required to prepare probes with high specific activity. This is extremely expensive and potentially dangerous. You can prepare radioactive RNA probes by using alkaline phosphatase to generate a 5'-hydroxyl end, followed by radioactive tagging using γ -³²P-ATP and T4 Polynucleotide Kinase, or by using α -³²P-(5',3')-biphosphate NDPs and ligating to the 3' end of the RNA using T4 RNA Ligase.

Q: What is the difference between RNA made with the DuraScribe® T7 Transcription Kit and RNA made with other *in vitro* transcription kits?

A: The DuraScribe T7 Transcription Kit produces RNA that contains nucleotides with a 2' ribose fluorine and that is resistant to degradation by A-type RNases (like the RNase found on human skin), while the AmpliScribe T7-Flash and other transcription kits make "standard" RNA. DuraScript™ RNA can be reverse-transcribed, like regular RNA, and can be digested by RNase III. However, DuraScript RNA cannot be used as a template to produce proteins by *in vitro* translation.