

Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

Questions about some of EPICENTRE's Enzymes

Q: Why are EPICENTRE's enzymes of such high quality?

A: EPICENTRE's enzymes are of extremely high quality because of our dedication to developing optimized purification methods and rigorous quality control testing. These objectives result in outstanding enzyme activity and long-term stability. EPICENTRE uses stringent, proprietary processes, which greatly improve enzyme purity and thus ensure reproducible performance.

Q: Will Tobacco Acid Pyrophosphatase (TAP) cut an ApppG mRNA cap?

A: Yes, Tobacco Acid Pyrophosphatase will digest this, and other capped RNAs with modified cap analogs. TAP cleaves the capped RNA transcript to a 5'-phosphate (α -pG-RNA), a free phosphate (β -p), and the residual cap nucleotide (γ -Ap).

Q: What is a good dilution buffer for TAP?

A: For long-term storage purposes, use TAP storage buffer (50% glycerol, 10 mM Tris-HCl [pH 7.5], 100 mM NaCl, 1.0 mM dithiothreitol, 0.1 mM EDTA, and 0.01% Triton™ X-100). For same-day use, dilute the enzyme in 1X TAP reaction buffer (10X reaction buffer is included with the enzyme). For additional information on TAP, please see page 23.

Q: What non-standard nucleotides can be incorporated into RNA using EPICENTRE's mutant T7 and SP6 R&DNA Polymerases?

A: T7 and SP6 R&DNA Polymerases have been used to incorporate 2'-deoxy, 2'-NH₂, 2'-F, 2'-OMe and 2'-N₃ dNTPs

into nascent RNA strands. EPICENTRE offers the DuraScribe™ T7 Transcription Kit to prepare 2' Fluorine-modified RNA transcripts, which are RNase A resistant. (For more information please go to www.epicentre.com/durascribe.asp).

Q: Can I completely substitute a specific rNTP in an RNA molecule using the mutant T7 or SP6 R&DNA Polymerase?

A: Sometimes. For example, modified RNAs can be made using 100% 2'-F-dUTP or 2'-F-dCTP, but the corresponding 2'-F-dATP and 2'-F-dGTP are incorporated somewhat less efficiently. The T7 and SP6 R&DNA Polymerases incorporate different modified NTPs at different rates.¹ Processivity and transcription yield are further compromised when two or more different dNTPs or modified NTPs are used. Increasing the reaction time and increasing the reaction temperature to 42°C can improve the yield of the resulting transcript. For more information on T7 and SP6 R&DNA Polymerase, please see Product Data Sheet, page 16.

Q: Do any of your thermophilic DNA polymerases have proofreading activity?

A: FailSafe™ PCR Enzyme Mix and MasterAmp™ Extra-Long DNA Polymerase Mix are enzyme blends that contain proofreading activity. The fidelities of EPICENTRE's polymerase blends are greater than three times that of standard *Taq* Polymerase.

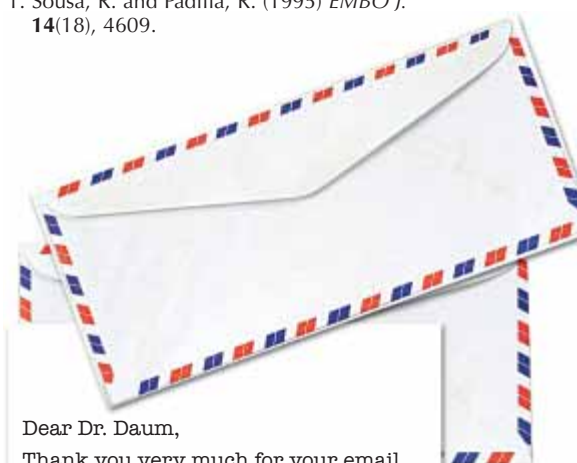
Q: Which of EPICENTRE's thermophilic DNA Polymerases add non-template A's on the 3'-end of the PCR product?

A: EPICENTRE's *Taq*, *Tfl*, *Tth* and AmpliTherm™ DNA Polymerases incorporate a non-template encoded A to the

3'-end of PCR products. The FailSafe PCR Enzyme Mix and MasterAmp Extra-Long DNA Polymerase Mix, result in a mixture of PCR products – some with an A and others without. Thus, PCR products made using FailSafe or MasterAmp Extra-Long Kits can be efficiently cloned in either a blunt-end or T/A cloning vector. For more information on EPICENTRE's DNA polymerases, please see Product Data Sheets, pages 11 and 12.

Reference

- Sousa, R. and Padilla, R. (1995) *EMBO J.* **14**(18), 4609.



Dear Dr. Daum,

Thank you very much for your email. It has always been a pleasure communicating with you. Your help and directions and in depth knowledge has opened new avenues for me. Research is always exciting but it is more so when you feel like a community.

I am really grateful for all the time and effort you took to extend your helpful hand. It only proves your generosity and the company culture.

Because of the nature of our research, I am sure we would be dealing more with your company in the future.

With many thanks and best regards,

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