



Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

Questions about FailSafe™ PCR Systems

Q: How does the FailSafe™ PCR System work?

A: The FailSafe PCR System is unique in that the optimal conditions for amplification are determined automatically by performing a single round of PCR, which is especially useful for solving tough PCR problems. The FailSafe™ PCR PreMix Selection Kit accomplishes this by using 12 different PCR PreMixes, which include buffer, deoxyribonucleotides, salt, and varying concentrations of magnesium salt and of the FailSafe™ PCR Enhancer (with betaine)*. The first time you use a specific template/primer combination, prepare PCR reactions using your template and primers with each of the 12 FailSafe™ PCR PreMixes. Analyze the PCR product from each PreMix reaction by agarose gel electrophoresis and visually determine which PreMix gives the best amplification: a single, strong PCR product of the desired length. Then use the selected PreMix for all subsequent amplifications with that template/primer combination. The product is called "FailSafe" because at least one of the 12 FailSafe PreMixes should give the desired product. Optimization is automatic.

Q: Can FailSafe be used for real-time (quantitative) PCR?

A: Yes! EPICENTRE has two unique FailSafe PCR Systems for real-time PCR. In both kits, the combination of the FailSafe™ PCR Enzyme Mix, the FailSafe PCR Enhancer and the optimized concentration of SYBR® Green I dye provide consistent and reproducible PCR efficiencies, which result in a broad dynamic range and some of the lowest possible threshold cycles. The FailSafe™ Real-Time PCR System is designed for well-based real-time PCR instruments and is very similar to the original FailSafe PCR

System (it uses 12 Real-Time PCR PreMixes, containing an optimal amount of SYBR® Green I dye). A separate tube of ROX dye is also provided as a reference standard for use in real-time PCR instruments manufactured by Applied Biosystems. The FailSafe™ Real-Time PCR Capillary System is designed specifically for capillary real-time PCR instruments and contains 8 PCR Capillary PreMixes for use with a smaller (20 µl) reaction volume. The optimization procedure for the FailSafe Real-Time PCR Systems is identical to the standard FailSafe PCR System - simply run PCR using each individual Real-Time PCR PreMix and select the best PreMix based on the lowest threshold cycle (C_T) and best melt curve.

Q: What DNA Polymerase is used in the FailSafe System?

A: The FailSafe PCR Enzyme Mix is a proprietary blend of thermostable DNA Polymerases, and includes a proofreading polymerase. This combination of enzymes provides excellent PCR efficiency and much higher fidelity than *Taq* DNA Polymerase, and allows amplification of even the toughest DNA templates. Regardless of the source or properties of the template, including those with high GC content, this enzyme mix efficiently amplifies the DNA.

Q: Can I use Topo or TA-cloning with the PCR product from the FailSafe PCR System?

A: Yes. The FailSafe PCR Enzyme Mix adds an uncoded "A" to both ends of many of the PCR products, and thus can be used in TA-cloning applications using T-vectors and also with Topo-TA cloning products.

Q: Which FailSafe PreMix should I use for my GC-rich template? I don't want to test all of the PreMixes.

A: It is nearly impossible to determine, without experimentation, which FailSafe PCR PreMix will work best with a given template/primer combination. We strongly recommend using all of the PreMixes for optimization. If only selected PreMixes are tested, you take the chance of selecting PCR conditions that may not be optimal for your reaction.

Q: I want to amplify a template of about 20 kb. Should I use the FailSafe PCR System or the MasterAmp™ Extra-Long PCR Kit?

A: Both products can amplify templates around 20 kb. With FailSafe, for templates >10 kb, we recommend using 2.5 Units of the FailSafe PCR Enzyme Mix (for templates <10 kb, use 1.25 units in 50-µl reactions). For any template over 20 kb use the MasterAmp™ Extra-Long PCR System.

**Patents issued and pending.*

Dear Hank,
Thank you very much for your time today - I greatly appreciate your help and advice. The detail on the email is wonderful! I will order the Selection Kit straight away and look forward to emailing you a photo of the results!

Kirsten

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