# The FailSafe™ Real-Time PCR System with SYBR® Green I Dye Provides Shorter Cycle Times and More Consistent PCR Quantitation with Every Template Every Time

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The new FailSafe™ Real-Time PCR System for quantitative PCR applications expands the functionality of the popular FailSafe™ PCR System. The FailSafe Real-Time PCR System incorporates SYBR® Green I dye for the detection and quantification of PCR products without the expense of labeled probes. Just like the standard FailSafe™ PCR System, this new real-time kit ensures successful quantitative PCR with every template the first time and every time.

The FailSafe Real-Time PCR System uses the FailSafe™ PCR Enzyme Mix, a unique blend of thermostable enzymes that is capable of amplifying the most difficult DNA templates with extremely high sensitivity and high fidelity. In addition, the patented FailSafe™ PCR Enhancer (with betaine)* greatly improves the specificity and consistency of PCR.

The other key components of the system are 12 unique FailSafe™ PCR PreMixes. Each FailSafe PCR PreMix contains everything else you need for a successful quantitative PCR except your own template and primers: SYBR® Green I dye, dNTPs, buffer, and varying amounts of $MgCl_2$ and FailSafe PCR Enhancer. A separate container of ROX, a fluorescent passive reference dye that is required for signal normalization in SYBR® Green I dye reactions assayed using ABI real-time PCR instruments, is also provided.

In this report, we compare the performance of the FailSafe Real-Time PCR System with real-time PCR kits from other major manufacturers. As a follow up to our report in *EPICENTRE Forum*, Volume 10, Number 1, which involved amplification of a relatively difficult DNA template (human Duchenne Muscular Dystrophy exon 43), we compare the kits from six suppliers (including EPICENTRE) for real-time amplification of a less complex DNA template (lambda *cII* gene). Comparison of data from different suppliers with an easily amplified template allows a more comparable, side-by-side analysis of real-time PCR results.

## Methods and Results

***FailSafe Real-Time PCR System consistently yields more specific and sensitive PCR data than all other kits tested with both difficult and routine templates***

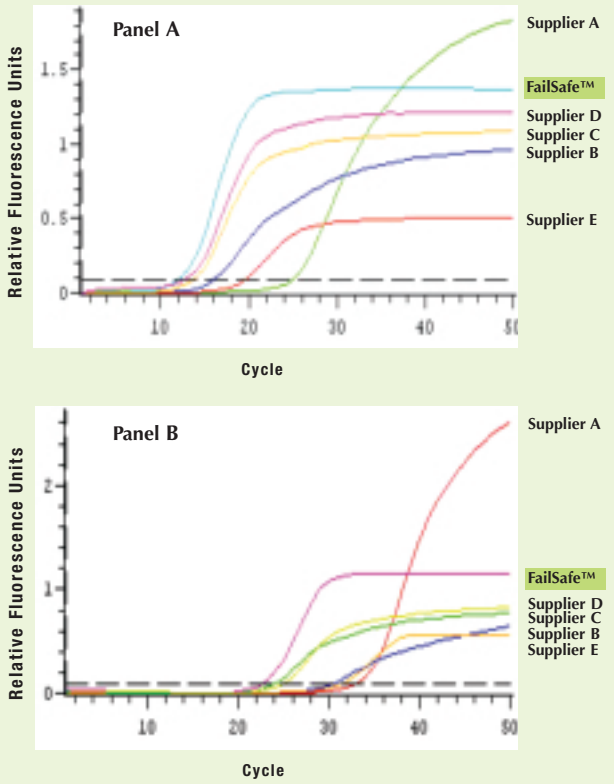



**Figure 1. Real-Time PCR quantification graphs comparing FailSafe™ Real-Time PCR System to five competitors' kits.** Panel A, Lambda *cII* gene amplification. Panel B, Human DMD 43 amplification. The Supplier A reaction contains a higher concentration of SYBR® Green I dye, which registers higher fluorescence signals, but not an increase in PCR products. A high concentration of SYBR® Green I dye actually inhibits the PCR reaction and results in a delayed cycle threshold.

To examine how the FailSafe Real-Time PCR System compares with 5 competitors' kits, a 460-bp fragment from the lambda *cII* gene and a 357-bp fragment of human Duchene Muscular Dystrophy exon 43 (DMD 43) were amplified using all six real-time PCR kits. The PCR reactions included: 50 pg of lambda DNA for *cII* amplification or 100 ng of human genomic DNA for DMD 43 amplification, 500 nmole each of the forward and reverse primers, and components of either the FailSafe™ Real-Time PCR System or a competitor's kit, according to the manufacturer's directions. FailSafe™ PreMix E was found to be the optimum PreMix in the first round of PCR for both amplicons and was used in the subsequent FailSafe™ Real-Time PCR reactions with these specific template/ primer sets. All reactions were set up at room temperature. Prior to thermocycling, competitors' hot-start enzymes

**Table 1. Cycle Threshold ($C_T$) Values comparing FailSafe™ Real-Time PCR System to five competitors' kits.** Panel A, Lambda *cII* gene amplification. Panel B, Human DMD 43 amplification. A lower $C_T$ value indicates more efficient amplification.

**Panel A**

| Supplier | $C_T$ Value (Average of Triplicate Reactions) |
|---|---|
| FailSafe™ Real-Time PCR System | 12.2 |
| Supplier A | 25.0 |
| Supplier B | 16.2 |
| Supplier C | 13.8 |
| Supplier D | 12.8 |
| Supplier E | 19.6 |

**Panel B**

| Supplier | $C_T$ Value (Average of Triplicate Reactions) |
|---|---|
| FailSafe™ Real-Time PCR System | 22.6 |
| Supplier A | 33.0 |
| Supplier B | 30.6 |
| Supplier C | 24.1 |
| Supplier D | 24.7 |
| Supplier E | 31.5 |

were reactivated according to each manufacturer's instructions. The FailSafe™ System does not use a hot-start enzyme and required no reactivation step. PCR cycling conditions were 50 cycles of 95°C for 10 seconds, 55°C for 10 seconds, and 72°C for 30 seconds.

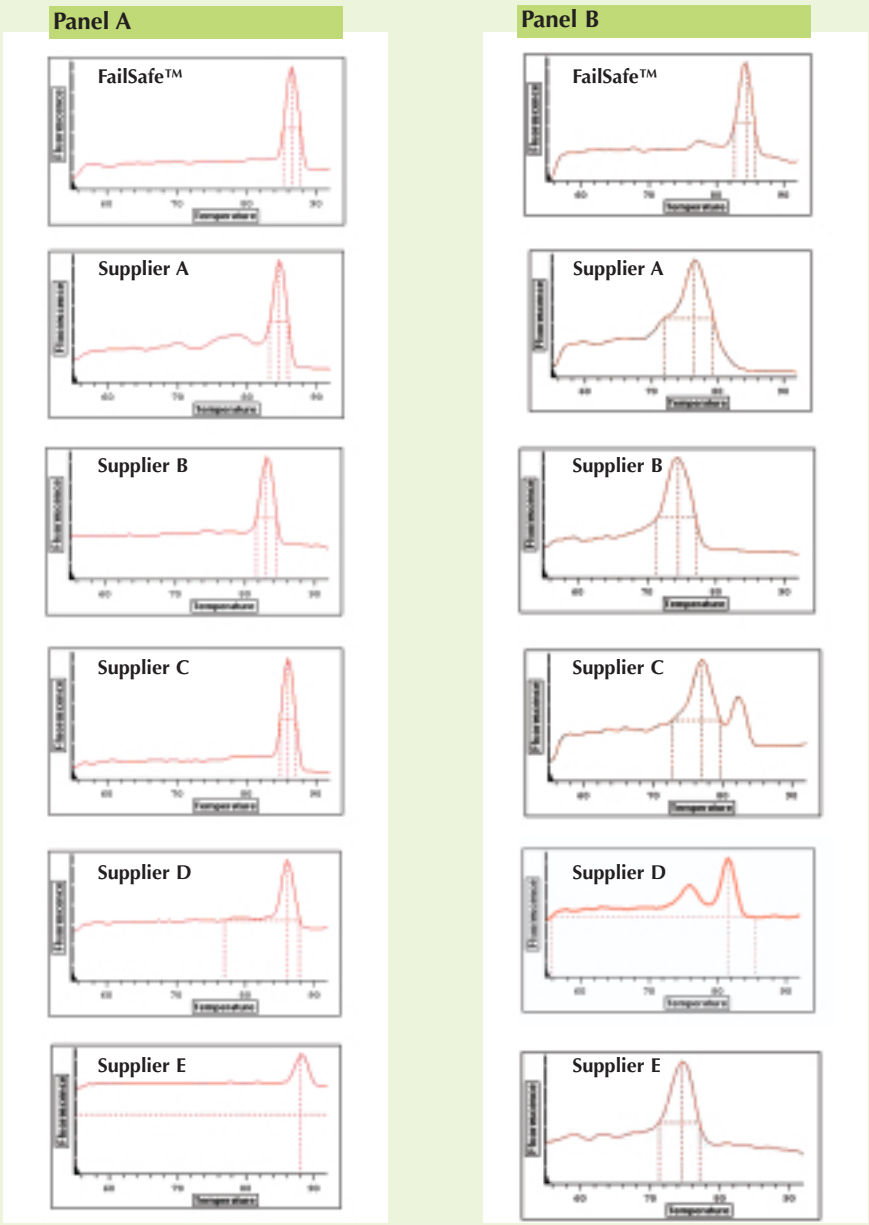
Figure 1 shows PCR quantification graphs for the two DNA templates and all six kit suppliers. Similarly, Figure 2 displays melt curve analyses of the same templates and kits. Table 1 shows comparative cycle threshold ($C_T$) values. In the Figures and Table, Panels A show real-time PCR results of lambda *cII* amplification and Panels B show results of DMD 43 amplification.

As shown in Figure 2, Panel A, all 6 real-time PCR kits worked well for lambda *cII* amplification. All graphs exhibit good peak symmetries and little or no primer-dimer formation. Upon examination of the $C_T$ values (Figure 1, Panel A and Table 1, Panel A) the FailSafe™ Real-Time PCR System performed equally well or better than the competition for real-time amplification of the lambda *cII* template.

When comparing the DMD exon 43 real-time PCR data however, the FailSafe Real-Time Kit gave significantly better $C_T$ values (see Figure 1, Panel B and Table 1, Panel B). The melt curve analysis graphs (Figure 2, Panel B) demonstrate a very specific, desired PCR amplicon for the FailSafe Real-Time Kit. Three of the competitors' kits only produced primer-dimers (Suppliers A, B, E). The other two had the specific PCR amplicon, but with undesirable formation of primer-dimers (Suppliers C and D).

For routine real-time PCR experiments, lambda *cII* DNA templates worked well with real-time PCR kits from most suppliers, but FailSafe™ worked better as revealed by lower $C_T$ values. With difficult real-time PCR amplifications (e.g., human DMD exon 43), most of the competitors' kits did not perform well, while the FailSafe Real-Time System demonstrated higher sensitivity and specificity. Superior amplification performance is due to several factors including flexible optimization of reaction conditions, a unique enzyme blend, and the patented FailSafe PCR Enhancer (with betaine).

**Figure 2. Melt curve analysis graphs comparing the FailSafe™ Real-Time PCR System to five competitors' kits.** Panel A, Lambda *cII* gene amplification. Panel B, Human DMD 43 amplification.




## Conclusions

The FailSafe Real-Time PCR System provides consistent, highly specific and sensitive quantitative data when compared to kits from five major suppliers. Our experimental data indicate excellent results with templates that are both difficult (human DMD exon 43) and routine (lambda *cII* gene). The reported quantitative PCR analyses were performed on MJ Research's Opticon® 2 instrument. Similar results were obtained using other instruments, including BioRad's iCycler iQ and ABI's Prism 7700.

**www.epicentre.com/realtimepcr.asp**

**FailSafe™ Real-Time PCR PreMix Selection Kit**

FSR0360 48 Reactions

***Contents:***

FailSafe™ PCR Enzyme Mix, 12 FailSafe™ Real-Time PCR 2X PreMixes, and Passive Reference Dye.

**FailSafe™ Real-Time PCR System**

FSR03200 200 Reactions

***Contents:***

FailSafe™ PCR Enzyme Mix, choice of two FailSafe™ Real-Time PCR 2X PreMixes, and Passive Reference Dye.

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This product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) and RT-PCR for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or purchased, i.e., an authorized thermal cycler.