



## Introducing TAQurate™ Real Time PCR Master Mix, A Single High-Performance Mix

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### Introduction

Real-Time PCR (quantitative PCR, qPCR) measures the amount of PCR product generated over time and is relative to the amount of template in the reaction. The technique is used to compare gene expression levels in different tissues, determine viral and bacterial loads, and validate microarray results for gene expression studies. Many of these applications are being done in high throughput environments, amplifying similar templates with routine sets of primers.

EPICENTRE's new TAQurate™ Real-Time PCR Master Mix provides successful real-time PCR with most routine templates and primer pairs. TAQurate Master Mix contains SYBR® Green I Dye, the TAQurate™ Real-Time PCR Enzyme Blend, and our patented PCR Enhancer (with betaine\*), in addition to optimized buffer, salts, and dNTP concentrations.

The TAQurate enzyme blend provides high sensitivity and specificity, and reliable amplification of even difficult templates. The PCR Enhancer (with betaine\*) ensures high amplification efficiencies and fewer nonspecific PCR products by stabilizing the DNA polymerase and reducing pauses and stops, even in troublesome GC-rich template regions.<sup>1</sup>

To use the TAQurate Master Mix, simply add the primers and template, mix thoroughly, and begin PCR. The ready-to-use TAQurate Master Mix saves setup time and reduces liquid-handling steps. This report demonstrates the dynamic range, sensitivity, and specificity of real-time PCR performed with TAQurate Real-Time PCR Master Mix, and compares the results with a PCR master mix from another leading supplier (Supplier A).

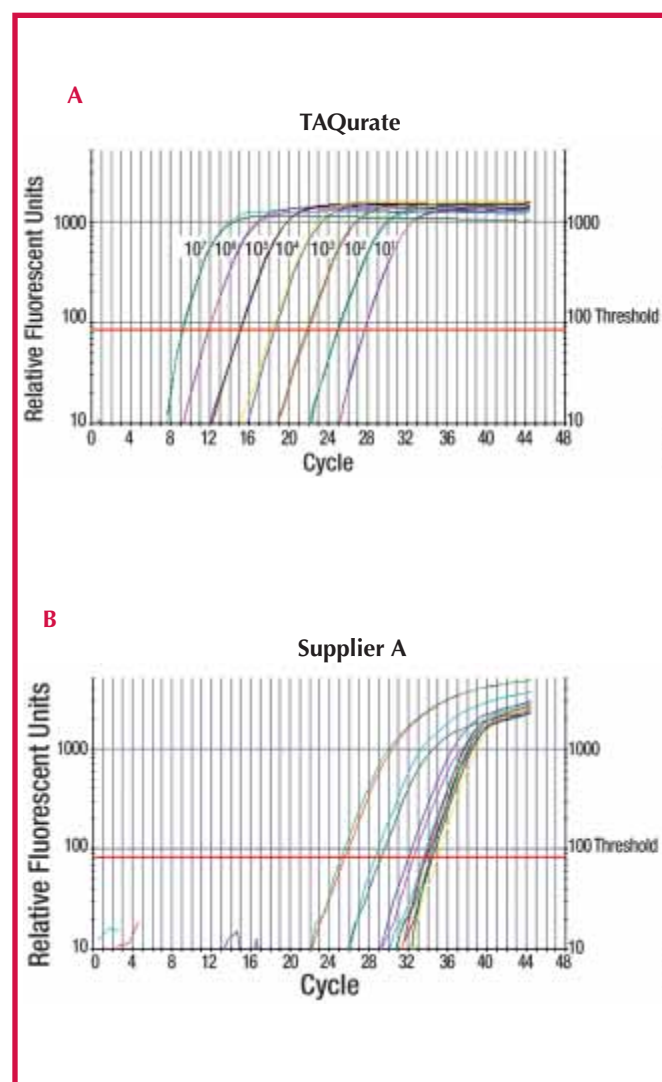
### Broad dynamic range, more accurate standard curve

To allow a more equitable comparison between the TAQurate Master Mix and Supplier A's master mix, lambda DNA, a relatively easy template to amplify,<sup>2</sup> was used to evaluate the dynamic range. Duplicate reactions containing 10, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup>, 10<sup>6</sup>, or 10<sup>7</sup> copies of lambda DNA were set up at room tem-

perature. To ensure a uniform distribution and fair comparison, distilled water, primers for the lambda *cII* gene (460-bp amplicon) and template DNA were mixed together and then aliquoted into the master mixes from both EPICENTRE and Supplier A. Supplier A's reactions were hot-start activated according to the manufacturer's recommendations. TAQurate Master Mix does not require a hot start. Reactions were amplified with an initial denaturation at 95°C (2 minutes), followed by 45 cycles of 94°C (30 seconds), 55°C (30 seconds) and 72°C (30 seconds), and analyzed using the BioRad iCycler iQ™ Real-Time PCR Detection System.

### Standard curve results

As indicated in Figure 1, the TAQurate Master Mix generated good quantification graphs over a dynamic range of 7 orders of magnitude. Plotting the log of the template concentration versus the threshold cycle ( $C_T$ ) generates a standard curve, which for the TAQurate Master Mix gave a 0.999 correlation coefficient. Amplification efficiency is derived from the slope of the standard curve ( $[10^{(-1/\text{slope})} - 1] \times 100$ ), which for TAQurate Master Mix gave a 105.8% amplification efficiency. The accepted amplification efficiency range is 90% to 110%. Over the dynamic range used here, PCR results with Supplier A's master mix gave a 0.831 correlation coefficient and 493.9% amplification efficiency, val-

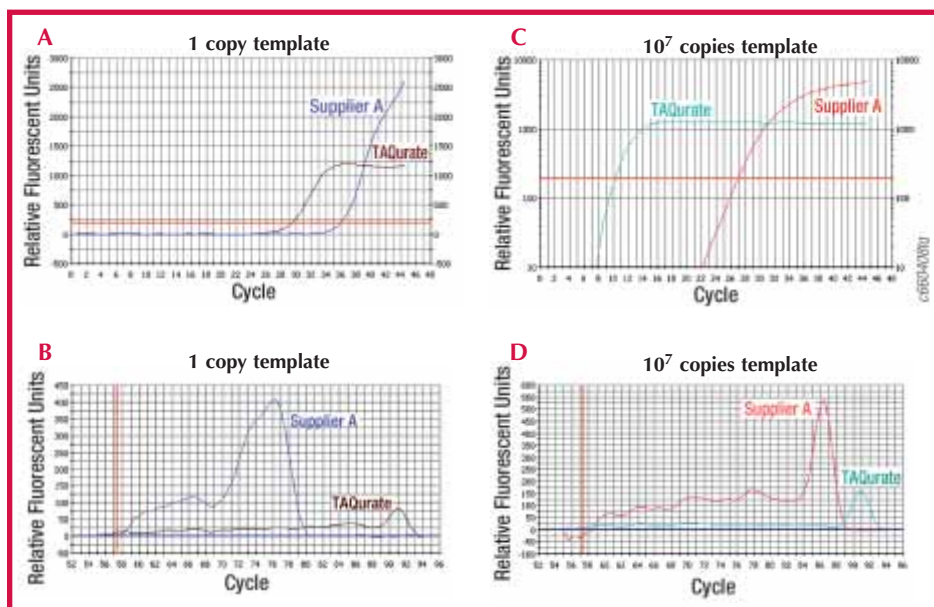


**Figure 1. Wider dynamic range generates a more accurate standard curve with the TAQurate™ Real-Time PCR Master Mix.** Real-time PCR of lambda DNA (10, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup>, 10<sup>6</sup> and 10<sup>7</sup> copies) was performed with TAQurate Real-Time PCR Master Mix and a master mix from another leading supplier (Supplier A). **A** (EPICENTRE) and **B** (Supplier A) PCR quantification graphs.

ues which are not acceptable for a useful standard curve.

### High sensitivity, faster $C_T$ values

Faster (lower)  $C_T$  values indicate greater PCR sensitivity. To compare the sensitivity of TAQurate Master Mix to Supplier A's master mix, reactions containing one copy or 10<sup>7</sup> copies of lambda DNA were amplified using the same reaction condi-



**Figure 2. Higher sensitivity and faster threshold cycle ( $C_T$ ) values with the TAQurate™ Real-Time PCR Master Mix.** Real-time PCR amplification of one copy and  $10^7$  copies of lambda DNA was performed with the TAQurate Real-Time PCR Master Mix and a master mix from another leading supplier (Supplier A). **A** and **C** are quantification graphs for one and  $10^7$  copies, respectively. **B** and **D** are melt curves for one and  $10^7$  copies, respectively.

tions described above. With one copy of template DNA, the TAQurate reaction had a  $C_T$  of 29.5 cycles and the Supplier A reaction had a significantly slower  $C_T$  of 36 cycles (Figure 2A). Higher relative concentrations of SYBR® Green I dye in Supplier A's master mix result in a higher fluorescence signal, but can actually inhibit the reaction and contribute to the slower  $C_T$  value.<sup>3</sup> The melt curves in Figure 2B show formation of a specific PCR product in the TAQurate reaction, but only primer-dimers and non-specific PCR products in the Supplier A reaction.

With  $10^7$  copies of template DNA, the TAQurate reaction had a  $C_T$  of 11 cycles compared to the Supplier A reaction with a  $C_T$  of 27 cycles (Figure 2C). At this higher template concentration, both master mixes gave good melt curves (Figure 2D). The significantly faster  $C_T$  values for the TAQurate reactions, at both low and high template concentrations, indicate greater PCR sensitivity.

**Effective two-step real-time RT-PCR**

To determine how well the TAQurate Master Mix works with cDNA, as used in gene expression studies, human cDNA was prepared from 1 ng of HeLa cell total RNA using EPICENTRE's MMLV reverse transcriptase in a 100- $\mu$ l reaction. The human  $\beta$ -actin message was then amplified by real-time PCR, in triplicate 50- $\mu$ l reactions, using 1  $\mu$ l of the above HeLa cell cDNA, 25 pmole of the forward and

reverse primers, and either TAQurate or Supplier A's master mix. Supplier A reactions were hot-start activated; TAQurate reactions were not. Reactions were amplified with an initial denaturation at 95°C (2 minutes), and 40 cycles of 95°C (10 seconds), 60°C (10 seconds), and 72°C (30 seconds).

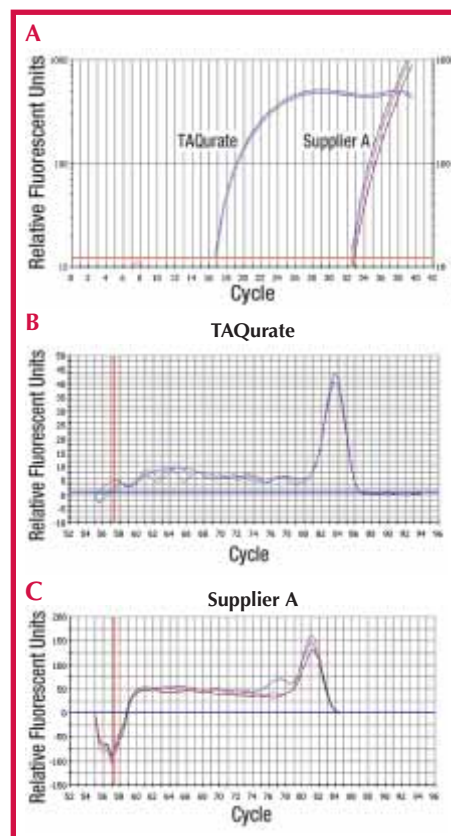
As indicated in Figure 3A, the TAQurate Master Mix reactions had an average  $C_T$  value of 19 compared to Supplier A's reactions with an average  $C_T$  value of 35. Both master mixes gave satisfactory melt curves, as shown in Figures 3B (EPICENTRE) and 3C (Supplier A). Using cDNA as the template, TAQurate Master Mix provides a more sensitive real-time PCR amplification.

**Conclusion**

EPICENTRE's new TAQurate Real-Time PCR Master Mix provides highly sensitive and specific real-time PCR amplifications for routine applications over a broad dynamic range. For more difficult templates, or to optimize real-time PCR conditions, EPICENTRE offers the FailSafe™ Real-Time PCR PreMix Selection Kit.<sup>2</sup>

**References**

1. Mytelka, D.S. and Chamberlin, M.J. (1996) *Nucleic Acids Res.* **24**(14), 2774.
2. Grunenwald, H. and Hunter, G.S. (2003) *EPICENTRE Forum* **10**(2), 14.
3. Nath, K. et al. (2000) *J. Biochem. Biophys. Methods* **42**(1-2), 15.



**Figure 3. Two-step real-time RT-PCR of human cDNA derived from HeLa cell total RNA was amplified with the TAQurate™ Real-Time PCR Master Mix and a master mix from another leading supplier (Supplier A).** **A**, quantification graphs; **B**, TAQurate melt curves; **C**, Supplier A melt curves.

[www.epicentre.com/taqurate.asp](http://www.epicentre.com/taqurate.asp)

<b>TAQurate™ Real-Time PCR Master Mix</b>	
TM046200	200 Reactions
<b>FailSafe™ Real-Time PCR PreMix Selection Kit</b>	
FSR0360	48 Reactions
<b>Contents:</b>	
FailSafe™ PCR Enzyme Mix, 12 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.