Thermo SCIENTIFIC

ABsolute™ Fast QPCR Master Mix: minimizing protocol time without compromising performance

With the ever-growing popularity of quantitative PCR (QPCR) as a sensitive technique for quantifying DNA and RNA, there is increasing demand for higher throughput of reactions and faster protocol times. ABsolute™ Fast QPCR Master Mixes have been optimized to deliver rapid QPCR results, without compromising the sensitivity and reproducibility of an assay.

QPCR is a technique used to amplify and simultaneously quantify the target sequence of a DNA molecule and has a wide range of applications in many biological disciplines[1]. It enables detection and quantification of gene expression, DNA copy number, viral titers, and transgene copy number as well as allelic discrimination, verification of microarray data and small interfering RNA knockdown validation. An increase in the use of this technique in recent years has boosted the demand for a higher throughput of reactions and a faster assay to reduce the overall protocol time. Thermo Scientific ABsolute Fast QPCR Master Mixes have been specially developed to give optimal results in a fraction of the time compared to standard QPCR mixes.

A standard QPCR reaction takes approximately 1 hour 45 minutes to complete. This includes an initial step of 15 minutes at 95 °C to activate the chemically modified hot-start *Taq* DNA polymerase, normally followed by 35–45 cycles of a 15-second denaturation at 95 °C and then 60 seconds annealing and extension at 60 °C. The activity of Taq DNA polymerase has been estimated to extend up to 1,000 base pairs in 60 seconds[2]. However, the majority of assays that are designed for QPCR have an amplicon size much lower than 1,000 base pairs and are normally 75–300 base pairs. There are situations in which amplicons greater than 300 base pairs are required, but these are exceptions to the rule and occur when design space is limited or applications downstream from QPCR require longer sequences. Therefore, to develop a fast QPCR assay, both the denaturation and annealing/extension steps could potentially be reduced to less than half the standard time without having to compromise the efficiency of *Taq* DNA polymerase. Moreover, the recent availability of reagents that can activate rapidly and perform optimally under fast thermal cycling conditions has also facilitated the development of fast QPCR master mixes. However, developing a fast QPCR assay has many challenges. In particular, the improvement in speed should not come at a loss of assay performance[3].

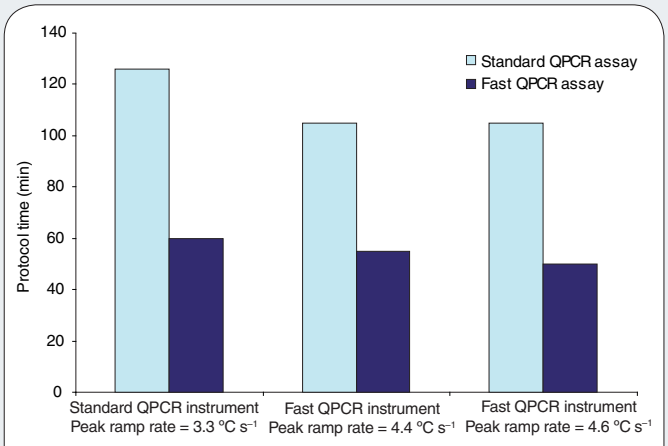

Figure 1 | Protocol time savings. Duration of a 45-cycle QPCR run with standard thermal cycling conditions (15 seconds at 95 °C and 60 seconds at 60 °C) and fast thermal cycling conditions (1 second at 95 °C and 20 seconds at 60 °C) using ABsolute Fast QPCR Master Mix and the indicated instruments. The polymerase activation step was reduced from 15 minutes required for the standard QPCR run to 5 minutes using ABsolute Fast QPCR Master Mix containing the new hot-start *Taq* DNA polymerase.

Thermo Fisher Scientific Inc. has launched ABsolute Fast QPCR Master Mixes for the rapid and reliable, probe-based detection of DNA and cDNA targets. ABsolute Fast QPCR Master Mix has been developed using Thermo-Fast, a new hot-start *Taq* DNA polymerase that requires an activation step at 95 °C of only 5 minutes (compared to 15 minutes required for a standard hot-start *Taq* DNA polymerase). The inclusion of this new hot-start *Taq* DNA polymerase in the ABsolute Fast QPCR Master Mixes allows rapid activation and specific amplification. Additional time savings can be achieved by substantially reducing the dwell times at both the denaturation and annealing/extension steps of the reaction. The reduction of these dwell times down to as low as 1 second at 95 °C followed by

Saima Naveed Nayab, Gerwyn Jones & Ian Kavanagh

Thermo Fisher Scientific, ABgene House, Blenheim Road, Epsom, KT19 9AP, UK.
Correspondence should be addressed to I.K. (ian.kavanagh@thermofisher.com).

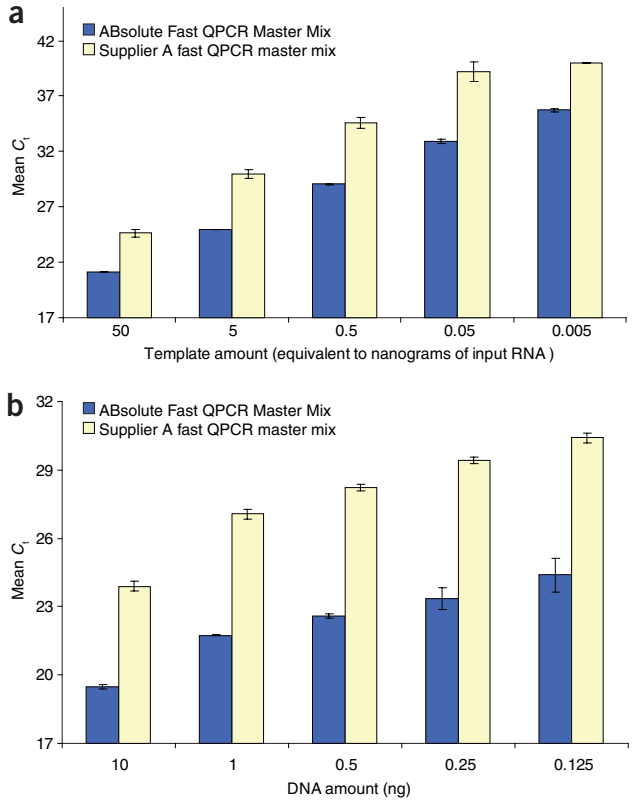

Figure 2 | Enhanced QPCR assay specificity and reproducibility. (**a**,**b**) Mean C_t values from three fast QPCR experiments performed in triplicate using tenfold serial dilutions (50 to 0.005 ng) of input human total liver RNA targeting a 226 base pair region of the *GAPDH* gene (**a**) and five dilutions (10, 1, 0.5, 0.25 and 0.125 ng) of *Saccharomyces cerevisiae* DNA amplifying 68 base pair region of the 18S rRNA gene (**b**). Performance of ABsolute Fast QPCR Master Mix was compared with Supplier A fast QPCR master mix under fast thermal cycling conditions (40 cycles of 1 second at 95 ˚C and 20 seconds at 60 ˚C). Error bars, s.d. calculated for 3 replicate QPCR assays run on separate plates.

20 seconds at 60 ˚C, coupled with a shorter enzyme activation step, allows completion of the QPCR protocol in less than 1 hour. A time savings of 50–60 minutes can be achieved without using a fast QPCR instrument (**Fig. 1**). Comparison of protocol time using both standard and fast instruments demonstrates that the only prerequisite for a rapid assay is a specially designed fast QPCR master mix.

ABsolute Fast QPCR Master Mixes have been optimized to deliver high-performance QPCR results on all major QPCR platforms without having any adverse effects on the specificity, efficiency, sensitivity and reproducibility of an assay. ABsolute Fast QPCR Master Mixes improve the performance of fast QPCRs when compared to alternative fast QPCR mixes, as judged by enhanced specificity, increased sensitivity (lower cycle threshold (C_t values), superior reproducibility of replicates and a more linear dynamic range (**Fig. 2**).

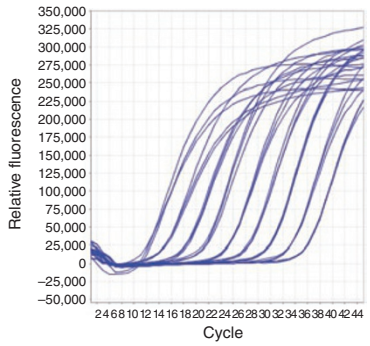

Figure 3 | ABsolute Fast QPCR Master Mixes deliver accurate and highly reproducible QRT-PCR data across a large template dynamic range. Amplification plots from a serial dilution (1×10^{-1} to 1×10^{-9}) of human cDNA (reverse transcribed from 1 µg of total liver RNA) using ABsolute Fast QPCR Master Mix. The assay targets a 68 base pair region of the 18S rRNA gene detected under fast thermal cycling conditions.

These ready-to-use reagents contain an inert blue dye. This dye, incorporated in the mix, increases the contrast between the reagent and plastic, thereby enabling visual confirmation of accurate pipetting. Consequently, ABsolute Fast QPCR Mixes are ideal for use with white QPCR plates, which reflect increased signal back to the detector for improved assay sensitivity. ABsolute Fast QPCR Master Mixes are compatible with any sequence-specific probe. In addition to accelerating QPCR analysis of DNA, the master mix can also be used for quantitative reverse-transcriptase PCR (QRT-PCR) analysis of RNA targets in conjunction with the Thermo Scientific Verso cDNA synthesis kit or any other reverse transcriptase kit. The two-step QRT-PCR results obtained using ABsolute Fast QPCR Master Mixes under fast thermal cycling conditions are extremely accurate and highly reproducible across a particularly large dynamic range (9 orders of magnitude) (**Fig. 3**).

With the current demand for higher throughput in QPCR applications, ABsolute Fast QPCR Master Mixes provide a perfect solution, delivering reliable results in a shorter time while maintaining confidence in high reproducibility, sensitivity and specificity.

1. Ginzinger, D. Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. *Exp. Hematol.* **30**, 503–512 (2002).
2. Lawyer, F.C. *et al.* High-level expression, purification, and enzymatic characterization of full-length *Thermus aquaticus* DNA polymerase and a truncated form deficient in 5′ to 3′ exonuclease activity. *PCR Methods Appl.* **2**, 275–287 (1993).
3. Hilscher, C., Vahrson, W. & Dittmer, D.P. Faster quantitative real-time PCR protocols may lose sensitivity and show increased variability. *Nucleic Acids Res.* **33**, e182 (2005).

This article was submitted to *Nature Methods* by a commercial organization and has not been peer reviewed. *Nature Methods* takes no responsibility for the accuracy or otherwise of the information provided.