Performance of a six-target multiplexing assay on the Azure Cielo™ 6 Real Time PCR Instrument using the Quantabio PerfeCTa® Multiplex qPCR ToughMix®

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Introduction

Real-time quantitative PCR (qPCR) is a powerful tool for gene expression analysis, pathogen detection, library quantification, SNP genotyping, and other applications. Multiplex qPCR assays can detect multiple targets in a single reaction, saving time and reducing sample requirements. The Quantabio PerfeCTa® Multiplex qPCR ToughMix® is a popular choice for qPCR assays, and we have evaluated its performance in a 6-target multiplex assay on the Azure Cielo™ 6 Real Time PCR instrument. Unlike other qPCR instruments, the Azure Cielo™ 6 Real Time PCR instrument does not require the use of a reference dye for background normalization, making it a true 6-channel qPCR system.

Materials and Methods

We used the Quantabio PerfeCTa® Multiplex qPCR ToughMix® (5X) (Quantabio, Beverly, MA, USA) with custom primers (IDT, Coralville, IA, USA; LGC Biosearch Technologies, Novato, CA, USA) to amplify six different genes in a multiplex qPCR assay: RRP36 (FAM), ACTB (HEX), SDHA (TAMRA), GAPDH (Texas Red), TBP (Cy 5), and B2M (Cy 5.5). The 20µL qPCR reaction volume consisted of 4µL of PerfeCTa® Multiplex qPCR ToughMix®, 0.25µL of each primer pair (20µM), 0.35µL of each probe (10µM), 2µL of pooled custom synthetic template (IDT, Coralville, IA, USA), and 10.4µL of nuclease-free water (Avantor, Radnor, PA, USA). A 5-point 10-fold dilution series (10^2 – 10^6 copies per reaction) of synthetic template was used to generate the standard curves. We used the following thermal cycling conditions on the Azure Cielo™ 6 Real Time PCR Instrument (Azure Biosystems, Dublin, CA, USA): 95°C for 3 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 90 sec. We performed all assays in triplicate, and we also included no-template controls (NTCs). All assays were prepared using white, low-profile, semi-skirted 96-well plates (VWR, Radnor, PA, USA) and optically clear sealing film (Azure Biosystems, Dublin, CA, USA). Data was analyzed in the Azure Cielo Manager analysis software (Azure Biosystems, Dublin, CA, USA).

Results

We found that the Quantabio PerfeCTa® Multiplex qPCR ToughMix® master mix performed well in the 6-target multiplex qPCR assay on the Azure Cielo™ Real Time PCR Instrument. The master mix provided robust amplification of all six targets with good sensitivity and specificity.

The six-plex multiplex assay using PerfeCTa® Multiplex qPCR ToughMix® generated sigmoidal-shaped amplification curves (Figures 1A–6A) for all the targets down to 100 copies tested in this assay. The standard curves (Figure 2A) of all six targets showed a linear correlation between the Cq values and the log copy number of the targets. The slopes of the standard curves were very close to 3.3 emphasizing that all the targets were amplified without any inhibition at different input levels of the target sample. None of the targets suffered any signal inhibition in the multiplex assay and the efficiency of each target was above 95%.

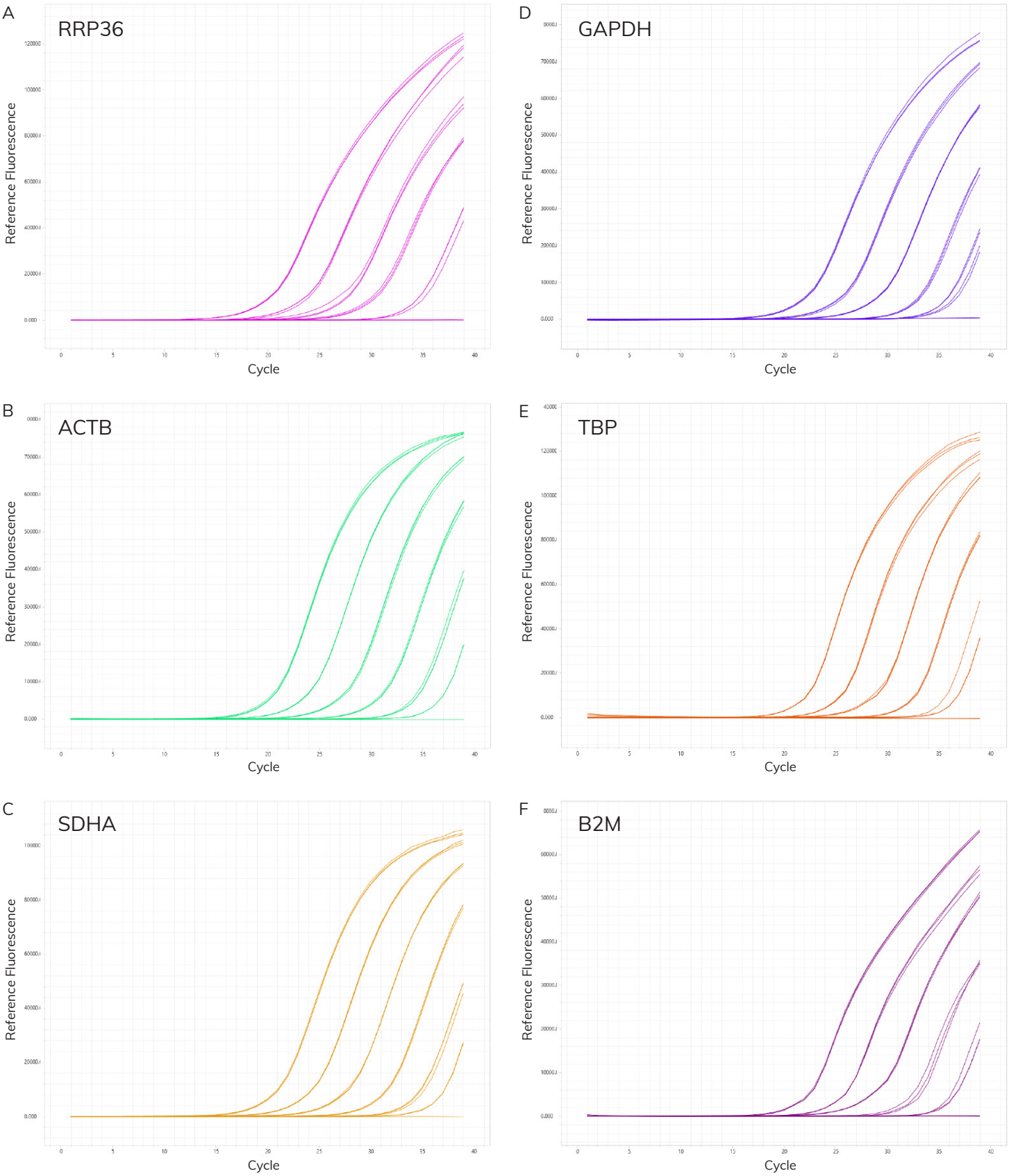
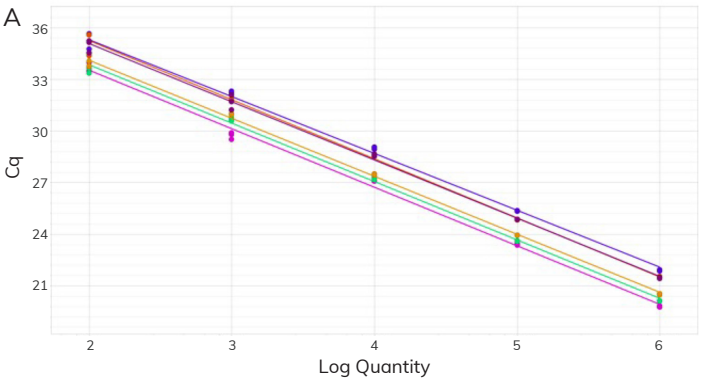


Figure 1. 10-fold dilutions of synthetic templates were added to the reaction mix and run in an Azure Cielo 6 Real-Time PCR System, collecting data in all 6 channels. Graph traces composed of three technical replicates at each dilution level are shown.

A



B

Target	Efficiency	Geometric Efficiency	R^2	Slope
RRP36	96.771	1.968	0.997	-3.402
ACTB	97.113	1.971	0.998	-3.393
SDHA	98.005	1.980	0.999	-3.371
GAPDH	100.775	2.008	0.995	-3.304
TBP	95.534	1.955	0.997	-3.434
B2M	97.508	1.975	0.997	-3.383

Figure 2. Standard curves derived from Cq data of 10-fold dilutions of synthetic templates. Linearity (R^2) and efficiency measurements are listed for each target.

Conclusion

Our results demonstrate that the Quantabio PerfeCTa® Multiplex qPCR ToughMix® master mix is an excellent choice for 6-target multiplex qPCR assays on the Azure Cielo™ Real Time PCR instrument. The master mix provides robust amplification of all targets with good sensitivity and specificity. The performance of this master mix makes it an ideal choice for researchers requiring reliable multiplex qPCR results.

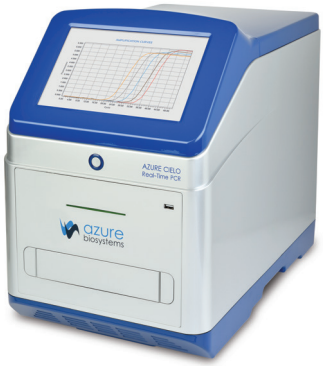


Figure 3. The Azure Cielo 6 Real-Time PCR (PN AIQ060) supports up to 6 channel multiplex qPCR reactions.

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