

Tech Note

qTOWER³ auto - Volume Independent Quantification

Accurate Quantification of qPCR Sample with Volumes ranging from 5 µL to 100 µL

Introduction

Reliable results and excellent data reproducibility are important factors when it comes to real-time PCR applications. Therefore, high quality qPCR reagents and master mixes are essential. However, the needed expense for these reagents can be balanced by considering using lower volumes. Another factor which needs to be considered in relation to the reaction volume is a limited amount of valuable samples or starting template. A simple scaling down of PCR reaction volumes, however, often poses a challenge with regards to comparability of results. Only qPCR thermal cyclers with optimal block homogeneity and temperature control accuracy, such as the qTOWER³ auto, are able to run a wide variety of sample volumes without any significant differences in final results.

Application

The detection of an *E. coli* K12 gene in two technical replicates and seven different qPCR reaction volumes. The experiment was completed using RT PCR Mix SYBR[®] C (A&A Biotechnology) and qTOWER³ auto.

Your Benefits

- Optimized for 0.2 mL consumables
- Ideal temperature control accuracy of ± 0.1 °C
- Excellent uniformity of ± 0.15 °C across the entire 96 well thermal block
- Four LEDs (RGBW) allow powerful excitation
- Patented fiber optic system guarantees identical detection of each well/tube without edge effects
- Suitable for sample volumes from 5 up to 100 µL

Results

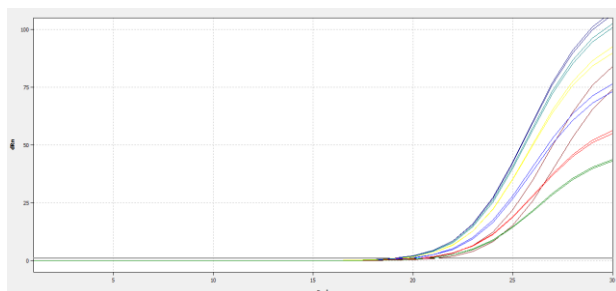


Figure 1: Amplification plots of all samples

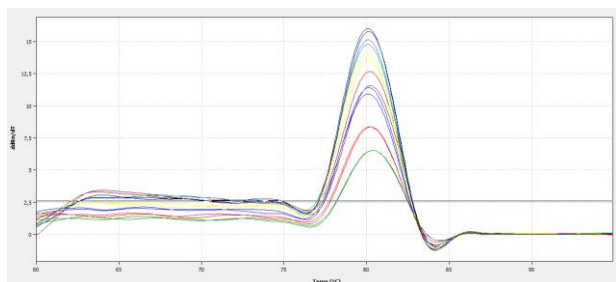


Figure 2: Melting curve analysis of all samples

Table 1: Analysis of Ct values and melting temperatures

Sample volume	Ct	SD (Ct)	Tm	SD (Tm)
2.5 µL (green)	20.7	0.07	80.4	0.00
5 µL (red)	20.34	0.001	80.2	0.00
10 µL (blue)	19.79	0.09	80.1	0.00
20 µL (yellow)	19.36	0.00	80.05	0.07
50 µL (dark blue)	19.08	0.01	80.1	0.00
80 µL (turquoise)	19.17	0.05	80.1	0.00
100 µL (brown)	20.81	0.08	80.2	0.00
Overall	19.76	0.56	80.1	0.03

Independent of the sample volume used for amplification of the *E. coli* K12 gene, Ct values are within a close range with slight variations at the lowest and highest used volumes going as low as 2.5 µL. However, the PCR product illustrated in the melting curve and temperatures show nearly identical results with overall low standard deviations. The qTOWER³ auto is a high-class qPCR thermal cycler assuring ideal amplifications in sample volumes from 5 µL to 100 µL.

Reference: TechNote_qTOWER³ auto_Volume independence_002_en.docx

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