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Tech Note
qTOWER³ 84 auto - Volume Independent Quantification

Accurate Quantification of qPCR Sample with Volumes ranging from 2 µL to 30 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 84 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 three technical replicates and ten different qPCR reaction volumes is demonstrated here. The experiment was completed using RT PCR Mix SYBR® C (A&A Biotechnology) and a qTOWER³ 84 auto.

Your Benefits

- Optimized for 0.1 mL consumables

- Ideal temperature control accuracy of ± 0.1 °C

- Excellent uniformity of ± 0.15 °C across the entire 384 well thermal block

- Patented fiber optic system guarantees identical detection of each well/tube without edge effects

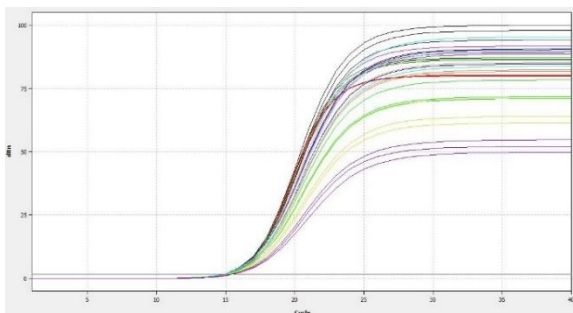- Suitable for sample volumes from 2 up to 30 µL
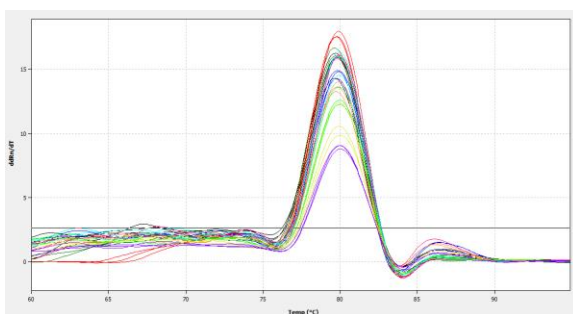
Results

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 µL (violet)	15.37	0.09	80.0	0.00
3 µL (yellow)	15.09	0.06	79.93	0.06
4 µL (light green)	15.00	0.05	79.97	0.06
5 µL (light blue)	14.67	0.07	79.93	0.06
7.5 µL (pink)	14.82	0.10	79.90	0.10
10 µL (orange)	14.86	0.01	79.8	0.10
12.5 µL (blue)	14.77	0.08	79.83	0.06
20 µL (red)	15.10	0.02	79.80	0.10
25 µL (green)	14.90	0.02	79.83	0.06
30 µL (black)	14.67	0.01	79.8	0.10
Overall	**14.93**	**0.17**	**79.88**	**0.07**

Independent of the sample volume used for amplification of *E. coli* K12 gene, Ct values are within a close range with a low standard deviation over all volumes of 0.17. Additionally, the PCR product illustrated in the melting curve and temperatures shows nearly identical results with overall low standard deviations. The qTOWER³ 84 auto is a high-class qPCR thermal cycler assuring ideal amplifications in sample volumes from 2 µL to 30 µL.