

Accurate Quantification of qPCR Samples 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 number 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 qTOWERiris, 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 qTOWERiris.

Your Benefits

- Suitable for sample volumes from 5 to 100 µL
- Ideal temperature control accuracy of ± 0.1 °C
- Excellent uniformity of ± 0.15 °C across the entire 96 well thermal block
- Patented fiber optic system guarantees identical detection of each well/tube without edge effects

Results

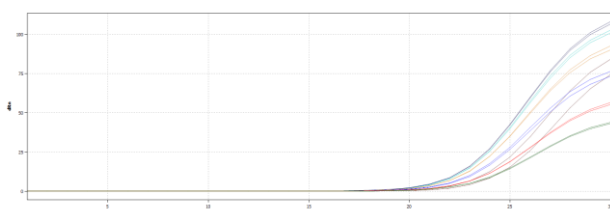


Figure 1: Amplification plots of all samples

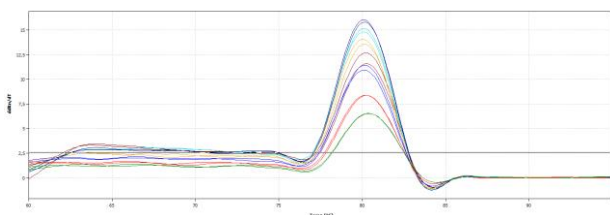


Figure 2: Melting curve analysis of all samples

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 qTOWERiris is a high-class qPCR thermal cycler assuring ideal amplifications in sample volumes from 5 µL to 100 µL.

Reference: TechNote_qTOWERiris_Volume independence_0013_en.docx; This document is true and correct at the time of publication; the information within is subject to change. Other documents may supersede this document, including technical modifications and corrections. © Analytik Jena GmbH+Co. KG

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