

Accurate Quantification of qPCR Sample with Volumes ranging from 2 μ L to 30 μ L

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 384, 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 qTOWERiris 384.

Your Benefits

- Suitable for sample volumes from 2 to 30 μ L
- Excellent uniformity of ± 0.15 $^{\circ}$ C across the entire 384 well thermal block
- Ideal temperature control accuracy of ± 0.1 $^{\circ}$ C
- Patented fiber optic system guarantees identical detection of each well/tube without edge effects

Results

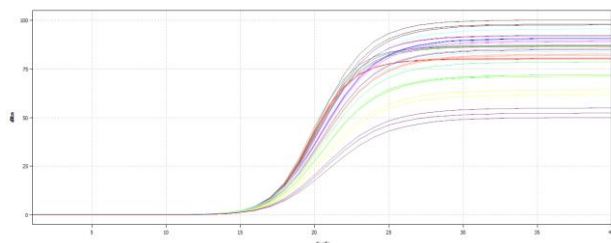


Figure 1: Amplification plots of all samples

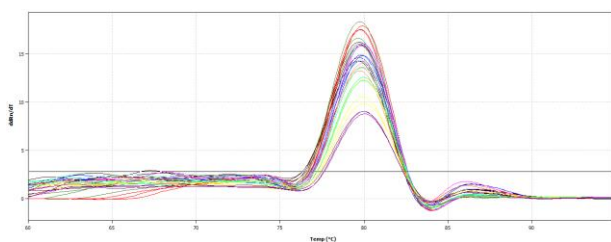


Figure 2: Melting curve analysis of all samples

Table 1: Analysis of Ct values and melting temperatures (Tm)

Sample volume	Ct	SD (Ct)	Tm	SD (Tm)
2 μ L (violet)	15.37	0.08	80.0	0.00
3 μ L (yellow)	15.03	0.06	79.93	0.06
4 μ L (light green)	14.92	0.06	79.97	0.06
5 μ L (light blue)	14.60	0.06	79.93	0.06
7.5 μ L (pink)	14.79	0.13	79.90	0.10
10 μ L (orange)	14.78	0.03	79.8	0.10
12.5 μ L (blue)	14.71	0.08	79.83	0.06
20 μ L (red)	15.14	0.02	79.80	0.10
25 μ L (green)	14.96	0.05	79.72	0.10
30 μ L (black)	14.69	0.15	79.7	0.08
Overall	14.90	0.07	79.84	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 0.07 (figure 1 & 2, table 1). Additionally, the PCR product illustrated in the melting curve and temperatures shows nearly identical results with overall low standard deviations. The qTOWERiris 384 is a high-class qPCR thermal cycler assuring ideal amplifications in sample volumes from 2 μ L to 30 μ L.

Reference: TechNote_qTOWERiris 384_Volume independence_0014_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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