

Tech Note

qTOWER³ - Volume Independent Quantification

Accurate Quantification of qPCR Sample Volumes from 5 µL to 80 µL

Introduction

Reliable results and excellent reproducibility are the most important topics of real-time PCR applications. Therefore, high qualitative qPCR reagents and master mixes, which are often associated with high costs, are essential. Another issue which must 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 regard to comparability of results. Only qPCR thermal cyclers with optimal block homogeneity and temperature control accuracy as well as ideal excitation and emission characteristics, such as the qTOWER³, are able to run a wide variety of sample volumes without any differences in final results.

Application

Detection of human genomic *sry* gene in three technical replicates and nine different qPCR reaction volumes. The experiment was completed using innuMIX qPCR MasterMix SyGreen and qTOWER³.

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 (up to 80 µL shown here)

Results

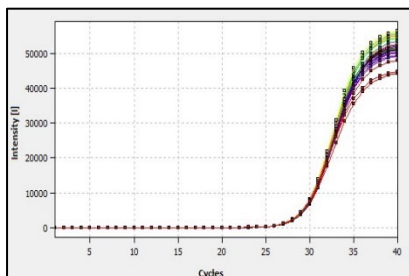


Figure 1: Amplification plots of all samples

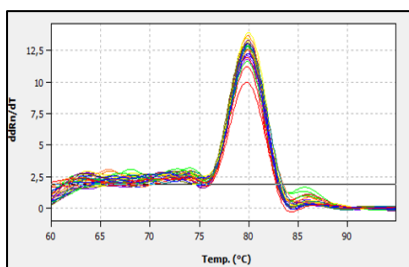


Figure 2: Melting curve analysis of all samples

Table 1: Analysis of Ct values and melting temperatures

Sample volume	Ct	SD (Ct)	Δ Ct	SD (T _m)
5 µL (red)	26.17	0.09	79.80	0.00
10 µL (violet)	26.19	0.04	79.83	0.06
20 µL (green)	26.18	0.04	79.90	0.00
30 µL (orange)	26.13	0.05	79.93	0.06
40 µL (yellow)	26.20	0.03	79.90	0.09
50 µL (turquoise)	26.25	0.05	79.97	0.06
60 µL (dark red)	26.29	0.08	79.87	0.06
70 µL (blue)	26.30	0.06	79.93	0.06
80 µL (purple)	26.26	0.01	79.77	0.06
Overall	26.22	0.07	79.88	0.08

Independent of the sample volume used for amplification of *sry* gene, Ct values as well as melting points show nearly identical results with overall standard deviations of 0.07 or 0.08 respectively. The qTOWER³ is a high-class qPCR thermal cycler assuring ideal amplifications in sample volumes from 5 µL to 80 µL.

Reference: TechNote_qTOWER³_Volume independence_001_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