Tech Note
qTOWERiris

Achieving Optimal Homogeneity in Real-time PCR with qPCR Master Mix for Probe-based Applications

Introduction

Real-time PCR, or qPCR, has become an indispensable technique in molecular biology for the detection and quantification of nucleic acids. This technology is crucial across various fields, including research and development, the pharmaceutical industry, quality assurance in food and animal feed, and forensic science, among others. As qPCR's significance grows, devices on the market are expected to meet increasingly stringent standards of quality and performance. Essential requirements include the capacity to support various analytical methods, the ability to detect multiple targets simultaneously in multiplex assays, and the need for highly sensitive and reproducible results. Achieving these standards largely depends on consistent excitation and detection across the entire sample block. This Tech Note shows how the qTOWERiris real-time qPCR thermal cycler meets these high standards for optimal measurement uniformity, demonstrated using the GoTaq® Probe qPCR Master Mix from Promega.

Your Benefits

- Patented high performance optical system of qTOWERiris series
- Optimal homogeneous excitation and detection in each of the 96 wells
- High reproducibility and Sensitivity

Application

A standard qPCR experiment with 96 samples in a 96 well PCR plate was performed with GoTaq® Probe qPCR Master Mix from Promega and a corresponding FAM-labelled probe in the real-time qPCR thermal cycler qTOWERiris. In the analysis, Ct values were considered as well as the homogeneity of the measurements based on the standard deviation of the Ct values and the deviation of the final fluorescence of all samples.

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Results

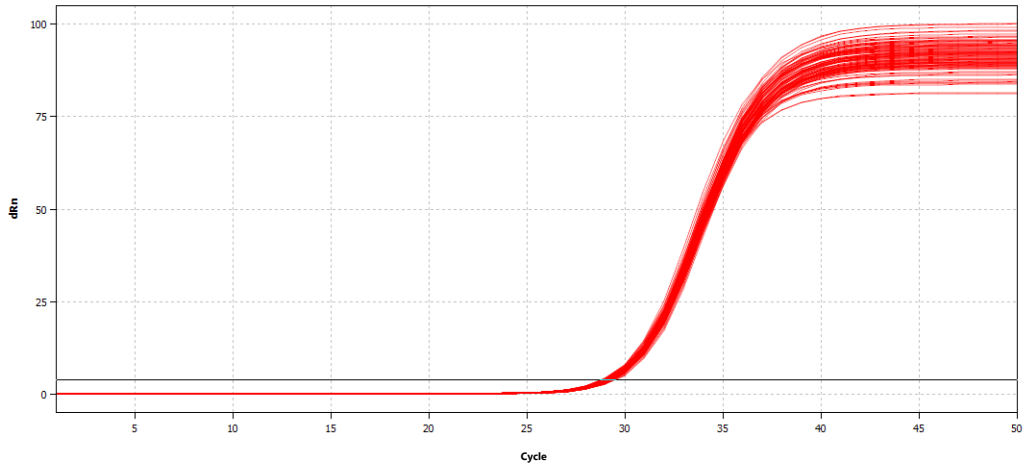

Figure 1: qPCR amplification curves of standard qPCR experiment over 96 samples

Table 1: Analysis data of qPCR amplification Ct: Cycle threshold; Std dev. Ct: Standard deviation of Ct.

Ct value	Std dev. Ct
29.15	0.14

Min. final fluorescence	Max. final fluorescence	Diff. final fluorescence	Deviation final fluorescence
31762	39131	7366	18.9%

Conclusion

Figure 1 clearly demonstrates the uniform distribution of amplification across the 96 samples in the standard probe-based qPCR experiment. The standard deviation of the Ct values, measured at 0.14, indicates a high degree of precision. Additionally, the deviation in final fluorescence across the 96 samples was 18.9%, well within the acceptable threshold of 30%. These results reflect the precise and homogeneous performance of the qTOWERiris using the GoTaq® Probe qPCR Master Mix from Promega.

Reference: TechNote_qTOWERiris_0020_Homogeneity Probe Master Mix_en.docx

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