Tech Note
qTOWERiris Series

analytikjena
An Endress+Hauser Company

Passive Reference in Real-time PCR

Introduction

Reliable and reproducible results in real-time PCR are prerequisite for trustworthy analysis in various application fields. In qPCR Thermocyclers of older generations, usually “corner effects” can introduce measurement errors, as the plate is not illuminated homogeneously. This leads to lower signals in wells that are furthest away from the light source in the middle of the plate. To overcome these effects “passive reference measurements” can be performed using ROX dye in the samples. In the Analytik Jena qTOWERiris Series, every well is excited and read out individually, which makes the passive reference measurement redundant. As many commercially available kits still have ROX dye as passive reference, it is possible to implement this measurement in the software, but it is not required.

Your Benefits

- Patented high performance optical system of qTOWERiris Series
- Optimal homogeneous excitation and detection in each of the 96 wells
- No need for passive reference measurement
- High reproducibility and sensitivity
- Maximum flexibility and constant results in different sample volumes

Application

In the experiment, samples were placed in the middle and all 4 corners of the plate, respectively. These were measured in 10 and 20 µL reaction volume to check, whether different sample heights also have an impact on the results. By using qTOWERiris, the GOI was measured in color module 1 using SYBR® Green as dye, while ROX dye was used as passive reference, measured in color module 4. For the review of possible corner effects, the PCR efficiency was considered in the different sample types.

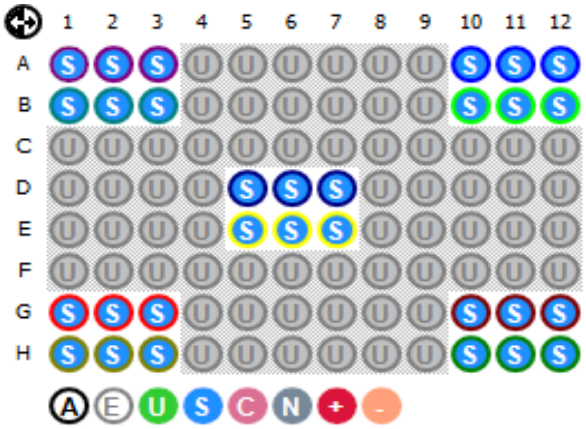

Figure 1: Sample layout

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Results

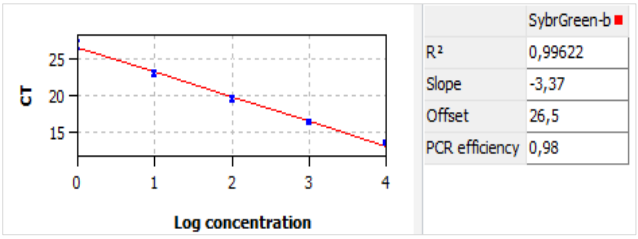

Figure 2: Standard curve 10 µL **with** passive reference

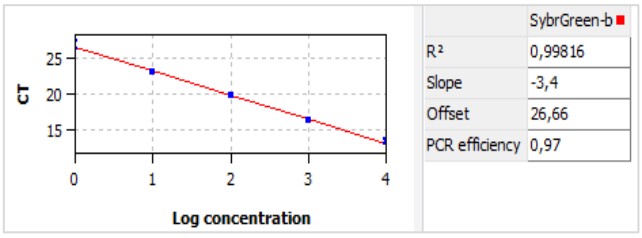

Figure 3: Standard curve 10 µL **w/o** passive reference

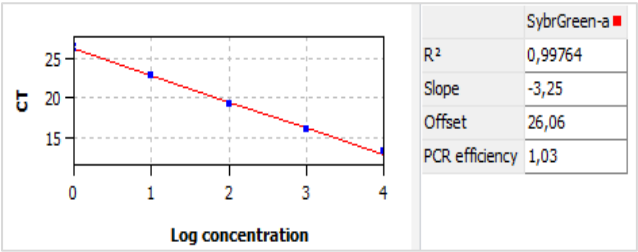

Figure 4: Standard curve 20 µL **with** passive reference

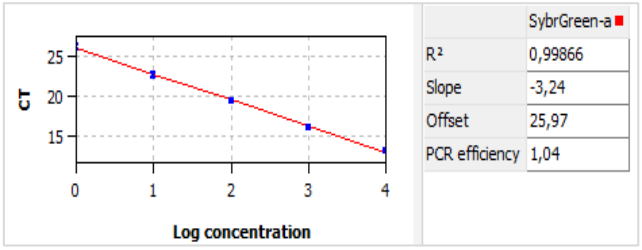

Figure 5: Standard curve 20 µL **w/o** passive reference

Considering the PCR efficiency in the standard curve, no considerable differences can be detected when passive reference is or is not selected in the software. There are also no effects of different sample volumes. Therefore, when using the qTOWERiris, it is not necessary to implement the ROX dye as a passive reference in every single experiment.

Reference: TechNote_qTOWERiris_0011_passive reference_en.doc

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