Assessment of Nucleic Acids with the Implen NanoPhotometer® According to MIQE Guidelines for qPCR

Quantitative real-time PCR (qPCR/RT-qPCR) has become indispensable in modern molecular biology labs. "However, such popularity created a myriad of different protocols, reagents, and analysis methods, which, when combined with different nucleic acid extraction and quality assessment methods, resulted in the publication of implausible and contradictory results."[1] To ensure standardized and reproducible protocols, MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) Guidelines have been introduced in 2009[1] and renewed in 2017[2] by Stephen Bustin *et al.* and compiled to a compendium by Afif Nour & Michael Pfaffl in 2020[3]. They provide guidance regarding necessary information for evaluating qPCR experiments – helping scientists to design best practice assays and reviewers to assess the validity of the protocols used.

Among various parameters listed in MIQE guidelines the following essential (E) and desirable (D) information can easily be obtained and verified using an Implen NanoPhotometer®:

- cDNA concentration (E)
- DNA or RNA quantification (E)
- Amount of cDNA/DNA (E)
- Contamination assessment (DNA or RNA) (E)
- Nucleic acid quantification (E)
- Amount of RNA (E)
- Purity (A_{260}/A_{280}) (D)

Using a sufficient and normalized template for concentration assessment is important as samples should lie within the standard curve and have similar signal strength to compare results and reduce errors during relative quantification or SNP genotyping. Sample QC is crucial as potential impurities might inhibit polymerase activity or inflate assumed template concentration leading to high Ct values and ultimately unreliable qPCR results. Compared to other technologies, the large concentration range starting from 1 - 16,500 ng/µl (dsDNA) is realized by two defined path lengths with fixed anchor points (True Path Technology™). By compressing the sample (Sample Compression Technology™) the NanoPhotometer® guarantees precise measurement free from evaporation even for samples with low surface tension. There are no additional efforts for costly recalibration or reconditioning required over the entire lifetime.

Integrated Blank and Sample Control software features of the Implen NanoPhotometer® warn the user if possible contaminations such as proteins, buffer salts, phenol, or guanidine as well as air bubbles or lint residues have been detected. With intuitive touchscreen operation, assessment of sample QC is done with just a few clicks and without extensive training needed. As sample volumes of only 0.3 - 2.0 µl are needed, assessing sample concentration and quality can be done between every error-prone step of qPCR workflows like nucleic acid extraction or cDNA transcription without sacrificing large sample volumes. This saves precious time and money if only the recent step has to be repeated or improved compared to trouble-shooting the entire range of experiments after qPCR has failed.

The Implen NanoPhotometer® is used in thousands of laboratories worldwide to quickly analyse a large number of samples prior to downstream applications providing valuable information in line with MIQE guidelines concerning concentration and quality. Visit www.implen.de to find out how the NanoPhotometer® can improve your research.

References

[1] Stephen A Bustin, Vladimir Benes, Jeremy A Garson, Jan Hellemans, Jim Huggett, Mikael Kubista, Reinhold Mueller, Tania Nolan, Michael W Pfaffl, Gregory L Shipley, Jo Vandesompele, Carl T Wittwer, The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments, Clinical Chemistry, Volume 55, Issue 4, 1 April 2009, Pages 611–622, https://doi.org/10.1373/clinchem.2008.112797

[2] Stephen A Bustin, Carl T Wittwer, MIQE: A Step Toward More Robust and Reproducible Quantitative PCR, Clinical Chemistry, Volume 63, Issue 9, 1 September 2017, Pages 1537–1538, https://doi.org/10.1373/clinchem.2016.268953

[3] Afif M. Abdel Nour & Michael W. Pfaffl, MIQE & qPCR:How to apply the MIQE Guidelines – a visual, interactive and practical qPCR guide!, 4th Edition, ISBN-9783000488061, 09 June 2020, https://www.gene-quantification.de/MIQE_qPCR_iBook_v4_June_2020.pdf