MIQE 2.0 Guidelines for Real-Time PCR: Checklist for Authors, Reviewers, and Editors

MIQE 2.0 modernizes the qPCR minimum reporting standards for today's digital, quantitative, high-throughput, and reproducibility-focused research environment. (Bustin, et al. 2025). The checklist below outlines best practices across the full workflow, from experimental design and sample handling to validation and data analysis, to reduce variability, strengthen reproducibility, and increase confidence in your qPCR data.

Item	Comments/My Information
Experimental Design	
Definition of experimental and control groups	
Number within each group	
Assay location; core or investigator's laboratory	
Acknowledgement of authors' contributions	
Samples	
Description	
Volume/mass of sample processed	
Microdissection or macrodissection	
Sample collection and storage	
Report sample collection procedures, storage, and transport conditions, freeze-thaw cycles, and handling procedures before nucleic acid extraction.	
Processing procedure	
If frozen, how and how quickly?	

Item	Comments/My Information
Nucleic Acid Extraction	
Procedure and/or instrumentation	
Kit name; details of any modifications	
Source of additional reagents used	
Details of DNase or RNase treatment	
Contamination assessment (DNA or RNA)	
Nucleic acid quantification	
Instrument and method	
Purity (A_{260}/A_{280})	
Yield	
RNA integrity method/instrument	
RIN/RQI or Cq of 3' and 5' transcripts	
Electrophoresis traces	
Inhibition testing (Cq dilutions, spike, or other)	
Reverse Transcription	
Complete reaction conditions	
Amount of RNA and reaction volume	
Priming oligo (if using GSP) and concentration	

Item	Comments/My Information
Reverse transcriptase and concentration	
Temperature and times	
Manufacturer of reagents and product codes	
Cqs with and without RT: convert Cq values into efficiency-corrected target quantities where appropriate. Report prediction intervals, thresholds, baseline settings, and data filtering criteria as noted in other fields below.	
Storage conditions of cDNA	
qPCR Target Information	
Gene symbol	
Sequence accession number	
Location of amplicon	
Amplicon length	
In silico specificity screen (BLAST, etc.)	
Pseudogenes, retropseudogenes, or other homologs?	
Sequence alignment	
Secondary structure analysis of amplicon	
Location of each primer by exon or intron (if applicable)	
What splice variants are targeted?	

Item	Comments/My Information
qPCR Oligonucleotides	
Primer sequences	
RTPrimerDB identification number	
Probe sequences	
Location and identity of any modifications	
Manufacturer of oligonucleotides	
Purification method	
qPCR Protocol	
Complete reaction conditions	
Reaction volume and amount of cDNA/DNA	
Primer (probe), Mg^{++}, and dNTP concentrations	
Polymerase identity and concentration	
Buffer/kit identity and manufacturer	
Exact chemical constitution of buffer(s)	
Additives (SYBR® Green I, DMSO, etc.)	
Plate/tube manufacturer product codes	
Describe template dilution procedures and explain how inhibition or matrix effects were evaluated.	

Item	Comments/My Information
Complete thermocycling parameters	
Reaction setup (manual/robotic)	
Manufacturer of qPCR instrument	
qPCR Validation	
Evidence of optimization (from gradients)	
Specificity (gel, sequence, melt, or digest)	
For SYBR® Green I, Cq of the NTC	
Standard curves with slope and y-intercept	
PCR efficiency calculated from slope	
Confidence interval for PCR efficiency or standard error	
R^2 of standard curve	
Linear dynamic range	
Cq variation at lower limit	
Confidence intervals throughout range	
Evidence for LOD	
If multiplex assay, efficiency and LOD of each assay	

Item	Comments/My Information
Data Analysis	
qPCR analysis program (source, version)	
Cq method determination	
Outlier identification and disposition	
Results of assay controls: no template controls (NTC), no amplification controls (NAC), no reverse transcriptase controls (NRT/MRT), positive controls, and extraction controls when applicable	
Justification of number and choice of reference genes	
Description of normalization method: include efficiency-corrected quantification, normalization against validated reference genes, and justification for the chosen approach	
Number and concordance of biological replicates	
Number and stage (RT or qPCR) of technical replicates: specify the number of biological replicates, technical replicates, independent experiments, and experimental units for each group	
Repeatability (intra-assay variation)	
Reproducibility (inter-assay variation, %CV)	
Power analysis	
Statistical methods for result significance: describe the statistical methods used to assess significance, assumptions tested, outlier handling, and multiple-comparison corrections, if applicable	
Software (source, version)	
Cq and raw data submission (Raw amplification data, Cq values, amplification curves, and metadata in supplemental files or public repositories)	

BLAST, Basic Local Alignment Search Tool; Cq, quantification cycle; CV, coefficient of variation; DMSO, dimethyl sulfoxide; FFPE, formalin-fixed paraffin-embedded; GSP, gene-specific primer; LOD, limit of detection; MIQE, minimum information for publication of quantitative real-time PCR experiments; NTC, no template control; qPCR, quantitative PCR; RDML, Real-Time PCR Data Markup Language; RDNA, residual DNA; RIN, RNA integrity number; RQI, RNA quality indicator; RT, reverse transcription; RTPrimerDB, freely accessible database and analysis tool for real-time quantitative PCR assays.

* Assessing the absence of DNA using a no–reverse transcription (no-RT) assay is essential when first extracting RNA. Once the sample has been validated as RDNA-free, the inclusion of a no-RT control is desirable but no longer essential.

** Disclosure of the probe sequence is highly desirable and strongly encouraged. However, since not all commercial predesigned assay vendors provide this information, it cannot be an essential requirement. Therefore, the use of assays that do not provide context sequences is not advised.

References

1. Bustin SA et al. (2025). MIQE 2.0: revision of the minimum information for publication of quantitative real-time PCR experiments guidelines. Clin Chem 71, 634-651.

Visit **bio-rad.com/MIQE_qPCRddPCR** for more information.

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