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

All essential MIQE information (E) must be submitted with the manuscript (see Bustin et al. 2009). Desirable information (D) should be submitted if available. If primers were obtained from RTPrimerDB, information on qPCR target, oligonucleotides, protocols, and validation is available from that source.

| Item | MIQE | Comments/My Information |
|---|---|---|
| **Experimental Design** | | |
| Definition of experimental and control groups | E | |
| Number within each group | E | |
| Assay location; core or investigator's laboratory | D | |
| Acknowledgement of authors' contributions | D | |
| **Samples** | | |
| Description | E | |
| Volume/mass of sample processed | D | |
| Microdissection or macrodissection | E | |
| Processing procedure | E | |
| If frozen — how and how quickly? | E | |
| If fixed — with what, how quickly? | E | |



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| Item | MIQE | Comments/My Information |
|---|---|---|
| Sample storage conditions and duration (especially for FFPE samples) | E | |
| **Nucleic Acid Extraction** | | |
| Procedure and/or instrumentation | E | |
| Kit name; details of any modifications | E | |
| Source of additional reagents used | D | |
| Details of DNase or RNase treatment | E | |
| Contamination assessment (DNA or RNA) | E | |
| Nucleic acid quantification | E | |
| Instrument and method | E | |
| Purity ($A_{260}/A_{280}$) | D | |
| Yield | D | |
| RNA integrity method/instrument | E | |
| RIN/RQI or Cq of 3' and 5' transcripts | E | |
| Electrophoresis traces | D | |
| Inhibition testing (Cq dilutions, spike, or other) | E | |
| **Reverse Transcription** | | |
| Complete reaction conditions | E | |
| Amount of RNA and reaction volume | E | |

| Item | MIQE | Comments/My Information |
|---|---|---|
| Priming oligo (if using GSP) and concentration | E | |
| Reverse transcriptase and concentration | E | |
| Temperature and times | E | |
| Manufacturer of reagents and product codes | D | |
| Cqs with and without RT | D* | |
| Storage conditions of cDNA | D | |
| **qPCR Target Information** | | |
| Gene symbol | E | |
| Sequence accession number | E | |
| Location of amplicon | D | |
| Amplicon length | E | |
| In silico specificity screen (BLAST, etc.) | E | |
| Pseudogenes, retropseudogenes, or other homologs? | D | |
| Sequence alignment | D | |
| Secondary structure analysis of amplicon | D | |
| Location of each primer by exon or intron (if applicable) | E | |
| What splice variants are targeted? | E | |

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| Item | MIQE | Comments/My Information |
|---|---|---|
| **qPCR Oligonucleotides** | | |
| Primer sequences | E | |
| RTPrimerDB identification number | D | |
| Probe sequences | D** | |
| Location and identity of any modifications | E | |
| Manufacturer of oligonucleotides | D | |
| Purification method | D | |
| **qPCR Protocol** | | |
| Complete reaction conditions | E | |
| Reaction volume and amount of cDNA/DNA | E | |
| Primer (probe), $Mg^{++}$, and dNTP concentrations | E | |
| Polymerase identity and concentration | E | |
| Buffer/kit identity and manufacturer | E | |
| Exact chemical constitution of buffer(s) | D | |
| Additives (SYBR® Green I, DMSO, etc.) | E | |
| Plate/tube manufacturer product codes | D | |
| Complete thermocycling parameters | E | |
| Reaction setup (manual/robotic) | D | |

| Item | MIQE | Comments/My Information |
| --- | --- | --- |
| Manufacturer of qPCR instrument | E | |
| **qPCR Validation** | | |
| Evidence of optimization (from gradients) | D | |
| Specificity (gel, sequence, melt, or digest) | E | |
| For SYBR® Green I, Cq of the NTC | E | |
| Standard curves with slope and y-intercept | E | |
| PCR efficiency calculated from slope | E | |
| Confidence interval for PCR efficiency or standard error | D | |
| $R^2$ of standard curve | E | |
| Linear dynamic range | E | |
| Cq variation at lower limit | E | |
| Confidence intervals throughout range | D | |
| Evidence for LOD | E | |
| If multiplex assay, efficiency and LOD of each assay | E | |
| **Data Analysis** | | |
| qPCR analysis program (source, version) | E | |
| Cq method determination | E | |
| Outlier identification and disposition | E | |

| Item | MIQE | Comments/My Information |
|---|---|---|
| Results of NTCs | E | |
| Justification of number and choice of reference genes | E | |
| Description of normalization method | E | |
| Number and concordance of biological replicates | D | |
| Number and stage (RT or qPCR) of technical replicates | E | |
| Repeatability (intra-assay variation) | E | |
| Reproducibility (inter-assay variation, %CV) | D | |
| Power analysis | D | |
| Statistical methods for result significance | E | |
| Software (source, version) | E | |
| Cq or raw data submission using RDML | D | |

* 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.

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.

## Reference

Bustin SA et al. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55, 611–622.

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