# The MIQE guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments

**MIQE checklist for authors, reviewers, and editors.**

<table>
<thead>
<tr>
<th>ITEM TO CHECK</th>
<th>IMPORTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXPERIMENTAL DESIGN</strong></td>
<td></td>
</tr>
<tr>
<td>Definition of experimental and control groups</td>
<td>E</td>
</tr>
<tr>
<td>Number within each group</td>
<td>E</td>
</tr>
<tr>
<td>Assay carried out by the core or investigator’s laboratory?</td>
<td>D</td>
</tr>
<tr>
<td>Acknowledgment of authors’ contributions</td>
<td>E</td>
</tr>
<tr>
<td><strong>SAMPLE</strong></td>
<td></td>
</tr>
<tr>
<td>Description</td>
<td>E</td>
</tr>
<tr>
<td>Volumes/measures of sample processes</td>
<td>D</td>
</tr>
<tr>
<td>Micro dissection or macrodissection</td>
<td>E</td>
</tr>
<tr>
<td>Processing procedure</td>
<td>E</td>
</tr>
<tr>
<td>If frozen, how and how quickly?</td>
<td>E</td>
</tr>
<tr>
<td>If fixed, what and how quickly?</td>
<td>D</td>
</tr>
<tr>
<td>Sample storage conditions and duration (especially for FFPE samples)</td>
<td>E</td>
</tr>
<tr>
<td><strong>NUCLEIC ACID EXTRACTION</strong></td>
<td></td>
</tr>
<tr>
<td>Procedure and/or instrumentation</td>
<td>E</td>
</tr>
<tr>
<td>Name and details of any modifications</td>
<td>E</td>
</tr>
<tr>
<td>Source of all reagents used</td>
<td>E</td>
</tr>
<tr>
<td>Details of DNA or RNA treatment</td>
<td>E</td>
</tr>
<tr>
<td>Contamination assessment (DNA or RNA)</td>
<td>E</td>
</tr>
<tr>
<td>Nucleic acid quantification</td>
<td>E</td>
</tr>
<tr>
<td>Instrument and method</td>
<td>E</td>
</tr>
<tr>
<td>Purify ((A_{260}/A_{280}))</td>
<td>D</td>
</tr>
<tr>
<td>Yield</td>
<td>Y</td>
</tr>
<tr>
<td>RNA integrity/method/instrument</td>
<td>E</td>
</tr>
<tr>
<td>RNAs or C(_{\text{q}}) of 3' and 5' transcripts</td>
<td>E</td>
</tr>
<tr>
<td>Electrophoresis tracts</td>
<td>D</td>
</tr>
<tr>
<td>Inhibition testing ((C_{\text{q}}) dilutions, spike, or other)</td>
<td>E</td>
</tr>
<tr>
<td><strong>REVERSE TRANSCRIPTION</strong></td>
<td></td>
</tr>
<tr>
<td>Complete reaction conditions</td>
<td>E</td>
</tr>
<tr>
<td>Amount of RNA and reaction volume</td>
<td>E</td>
</tr>
<tr>
<td>Priming oligonucleotide (if using dNTPs) and concentration</td>
<td>E</td>
</tr>
<tr>
<td>Reverse transcriptase and concentration</td>
<td>E</td>
</tr>
<tr>
<td>Temperature and time</td>
<td>E</td>
</tr>
<tr>
<td>Manufacturer of reagents and catalogue numbers</td>
<td>E</td>
</tr>
<tr>
<td>(C_{\text{q}}) with and without reverse transcription</td>
<td>D</td>
</tr>
<tr>
<td>Storage conditions of cDNA</td>
<td>D</td>
</tr>
<tr>
<td><strong>OLIGONUCLEOTIDES</strong></td>
<td></td>
</tr>
<tr>
<td>Gene symbol</td>
<td>q</td>
</tr>
<tr>
<td>Sequence accession number</td>
<td>E</td>
</tr>
<tr>
<td>Location of amplicon</td>
<td>D</td>
</tr>
<tr>
<td>Amplicon length</td>
<td>E</td>
</tr>
<tr>
<td>Intron-specific primer set (BLAST, and so forth)</td>
<td>E</td>
</tr>
<tr>
<td>Pseudogenes, retrotransposon, or other homologs?</td>
<td>D</td>
</tr>
<tr>
<td>Sequence alignment</td>
<td>D</td>
</tr>
<tr>
<td>Secondary structure analysis of amplicon</td>
<td>D</td>
</tr>
<tr>
<td>Location of each primer by exon or intron (if applicable)</td>
<td>E</td>
</tr>
<tr>
<td>What splice variants are targeted?</td>
<td>E</td>
</tr>
<tr>
<td><strong>PCR METHODS/TECHNIQUES</strong></td>
<td></td>
</tr>
<tr>
<td>Primer sequences</td>
<td>E</td>
</tr>
<tr>
<td>RT/Primer D B identification number</td>
<td>D</td>
</tr>
<tr>
<td>Probe sequences</td>
<td>D</td>
</tr>
<tr>
<td>Location and identity of any modifications</td>
<td>E</td>
</tr>
<tr>
<td>Manufacturer of oligonucleotides</td>
<td>D</td>
</tr>
<tr>
<td>Purification method</td>
<td>D</td>
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</tbody>
</table>

**PCR PROTOCOL**

<table>
<thead>
<tr>
<th>ITEM TO CHECK</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Complete reaction conditions</td>
<td>E</td>
</tr>
<tr>
<td>Reaction volume and amount of cDNA/RNA</td>
<td>E</td>
</tr>
<tr>
<td>Primer (probe), Mg(^{++}), and dNTP concentrations</td>
<td>E</td>
</tr>
<tr>
<td>Polymerase identity and concentration</td>
<td>E</td>
</tr>
<tr>
<td>Buffer identity and manufacturer</td>
<td>E</td>
</tr>
<tr>
<td>Exact chemical composition of the buffer</td>
<td>E</td>
</tr>
<tr>
<td>Additives (SYBR Green I, DMSO, and so forth)</td>
<td>D</td>
</tr>
<tr>
<td>Manufacturer of pipette tips and catalog number</td>
<td>E</td>
</tr>
<tr>
<td>Complete thermocycling parameters</td>
<td>E</td>
</tr>
<tr>
<td>Reaction setup (manual/robotic)</td>
<td>E</td>
</tr>
<tr>
<td>Manufacturer of qPCR instrument</td>
<td>D</td>
</tr>
</tbody>
</table>

**PCR VALIDATION**

<table>
<thead>
<tr>
<th>ITEM TO CHECK</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Evidence of optimisation (from gradients)</td>
<td>D</td>
</tr>
<tr>
<td>Specificity (g, sequence, melt, or dimer)</td>
<td>E</td>
</tr>
<tr>
<td>For SYBR Green (C_{\text{q}}), the NTC</td>
<td>E</td>
</tr>
<tr>
<td>Calibration curves with slope and y intercept</td>
<td>E</td>
</tr>
<tr>
<td>PCR efficiency calculated from slope</td>
<td>E</td>
</tr>
<tr>
<td>Ct for PCR efficiency or SE</td>
<td>E</td>
</tr>
<tr>
<td>(R^2) of calibration curve</td>
<td>Y</td>
</tr>
<tr>
<td>Linear dynamic range</td>
<td>E</td>
</tr>
<tr>
<td>(C_{\text{q}}) variation at LOD</td>
<td>E</td>
</tr>
<tr>
<td>Cycle threshold range</td>
<td>E</td>
</tr>
<tr>
<td>Evidence for LOD</td>
<td>D</td>
</tr>
<tr>
<td>% multiplex, efficiency and LOD of each assay</td>
<td>E</td>
</tr>
</tbody>
</table>

**DATA ANALYSIS**

<table>
<thead>
<tr>
<th>ITEM TO CHECK</th>
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</thead>
<tbody>
<tr>
<td>qPCR analysis program (source, version)</td>
<td>E</td>
</tr>
<tr>
<td>Method of (C_{\text{q}}) determination</td>
<td>E</td>
</tr>
<tr>
<td>Outlier identification and disposition</td>
<td>E</td>
</tr>
<tr>
<td>Results for NTCs</td>
<td>E</td>
</tr>
<tr>
<td>Justification of number and choice of reference genes</td>
<td>E</td>
</tr>
<tr>
<td>Description of normalisation method</td>
<td>E</td>
</tr>
<tr>
<td>Number and concordance of biological replicates</td>
<td>D</td>
</tr>
<tr>
<td>Number and stage (reverse transcription or qPCR) of technical replicates</td>
<td>E</td>
</tr>
<tr>
<td>Reproducibility (Intra assay variation)</td>
<td>E</td>
</tr>
<tr>
<td>Reproducibility (Interassay variation, CV)</td>
<td>E</td>
</tr>
<tr>
<td>Power analysis</td>
<td>D</td>
</tr>
<tr>
<td>Statistical methods for results significance</td>
<td>E</td>
</tr>
<tr>
<td>Software (source, version)</td>
<td>E</td>
</tr>
<tr>
<td>(C_{\text{q}}) or raw data submission with RDML</td>
<td>D</td>
</tr>
</tbody>
</table>

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*Information for this checklist has been adapted from the MIQE guidelines for publication of quantitative real-time PCR experiments. The Primerdesign MIQE checklist is available at www.primerdesign.co.uk and contact can be made via email at enquiry@primerdesign.co.uk or phone at +44 (0)23 8074 8830.

**Acknowledgment of authors’ contributions**

**EXPERIMENTAL DESIGN**

**SAMPLE**

**NUCLEIC ACID EXTRACTION**

**OLIGONUCLEOTIDES**

**PCR METHODS/TECHNIQUES**

**PCR PROTOCOL**

**PCR VALIDATION**

**DATA ANALYSIS**

**Summary**

The MIQE guidelines have been developed to ensure the transparency and reproducibility of quantitative real-time PCR experiments. The Primerdesign MIQE checklist is designed to provide a comprehensive list of the minimum information required to publish such experiments, ensuring that the results are both reliable and comparable. By adhering to these guidelines, researchers can enhance the quality and impact of their work, facilitating advancements in the field of molecular biology and beyond.