

# Are You MIQE\* Compliant?

\*Minimum Information for Publication of Quantitative Real-Time PCR Experiments

The MIQE Checklist for qPCR <sup>1</sup>	Importance <sup>2</sup>
<b>Experimental Design</b>	
Definition of experimental and control groups	Essential
Number within each group	Essential
Assay carried out by core lab or investigator's lab?	Desirable
Acknowledgement of authors' contributions	Desirable
<b>Sample</b>	
Description	Essential
Volume/mass of sample processed	Desirable
Microdissection or macrodissection	Essential
Processing procedure	Essential
If frozen - how and how quickly?	Essential
If fixed - with what, how quickly?	Essential
Sample storage conditions and duration (especially for FFPE samples)	Essential
<b>Nucleic Acid Extraction</b>	
Procedure and/or instrumentation	Essential
Name of kit and details of any modifications	Essential
Source of additional reagents used	Desirable
Details of DNase or RNase treatment	Essential
Contamination assessment (DNA or RNA)	Essential
Nucleic acid quantification	Essential
Instrument and method	Essential
Purity (A260/A280)	Desirable
Yield	Desirable
RNA integrity method/instrument	Essential
RIN/RQI or Cq of 3' and 5' transcripts	Essential
Electrophoresis traces	Desirable
Inhibition testing (Cq dilutions, spike or other)	Essential
<b>Reverse Transcription</b>	
Complete reaction conditions	Essential
Amount of RNA and reaction volume	Essential
Priming oligonucleotide (if using GSP) and concentration	Essential
Reverse transcriptase and concentration	Essential
Temperature and time	Essential
Manufacturer of reagents and catalogue numbers	Desirable
Cqs with and without RT	Desirable <sup>3</sup>
Storage conditions of cDNA	Desirable
<b>qPCR Target Information</b>	
If multiplex, efficiency and LOD of each assay.	Essential
Sequence accession number	Essential
Location of amplicon	Desirable
Amplicon length	Essential
In silico specificity screen (BLAST, etc)	Essential
Pseudogenes, retropseudogenes or other homologs?	Desirable
Sequence alignment	Desirable
Secondary structure analysis of amplicon	Desirable
Location of each primer by exon or intron (if applicable)	Essential
What splice variants are targeted?	Essential
<b>qPCR Oligonucleotides</b>	
Primer sequences	Essential
RTPrimerDB Identification Number	Desirable
Probe sequences	Desirable <sup>4</sup>
Location and identity of any modifications	Essential
Manufacturer of oligonucleotides	Desirable
Purification method	Desirable

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<b>qPCR Protocol</b>	
Complete reaction conditions	Essential
Reaction volume and amount of cDNA/DNA	Essential
Primer, (probe), Mg++ and dNTP concentrations	Essential
Polymerase identity and concentration	Essential
Buffer/kit identity and manufacturer	Essential
Exact chemical constitution of the buffer	Desirable
Additives (SYBR Green I, DMSO, etc.)	Essential
Manufacturer of plates/tubes and catalog number	Desirable
Complete thermocycling parameters	Essential
Reaction setup (manual/robotic)	Desirable
Manufacturer of qPCR instrument	Essential
<b>qPCR Validation</b>	
Evidence of optimization (from gradients)	Desirable
Specificity (gel, sequence, melt, or digest)	Essential
For SYBR Green I, Cq of the NTC	Essential
Standard curves with slope and y-intercept	Essential
PCR efficiency calculated from slope	Essential
Confidence interval for PCR efficiency or standard error	Desirable
R <sup>2</sup> of standard curve	Essential
Linear dynamic range	Essential
Cq variation at lower limit	Essential
Confidence intervals throughout range	Desirable
Evidence for limit of detection	Essential
If multiplex, efficiency and LOD of each assay.	Essential
<b>Data Analysis</b>	
qPCR analysis program (source, version)	Essential
Cq method determination	Essential
Outlier identification and disposition	Essential
Results of NTCs	Essential
Justification of number and choice of reference genes	Essential
Description of normalization method	Essential
Number and concordance of biological replicates	Desirable
Number and stage (RT or qPCR) of technical replicates	Essential
Repeatability (intra-assay variation)	Essential
Reproducibility (inter-assay variation, %CV)	Desirable
Power analysis	Desirable
Statistical methods for result significance	Essential
Software (source, version)	Essential
Cq or raw data submission using RDML	Desirable

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<sup>2</sup> All essential information must be submitted with the manuscript. Desirable information should be submitted if available. If primers are from RTPrimerDB, information on qPCR target, oligonucleotides, protocols, and validation is available from that source.

<sup>3</sup> Assessing the absence of DNA using a no-reverse transcription assay is essential when first extracting RNA. Once the sample has been validated as DNA-free, inclusion of no-reverse transcription control is desirable but no longer essential.

<sup>4</sup> Disclosure of the probe sequence is highly desirable and strongly encouraged. However, because not all commercial pre-designed assay vendors provide this information, it cannot be an essential requirement. Use of such assays is discouraged.

To learn more about MIQE, visit  
[sigma.com/miqe](http://sigma.com/miqe)