

Are You MIQE* Compliant?

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

The MIQE Checklist for qPCR		Importance
Experimental Design		
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
Sample		
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
Nucleic Acid Extraction		
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
Reverse Transcription		
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 ³
Storage conditions of cDNA		Desirable

qPCR Target Information	
If multiplex, efficiency and LOD of each assay	Essential
Sequence accession number	Essential
Location of amplicon	Desirable
Amplicon length	Essential
<i>In silico</i> 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
qPCR Oligonucleotides	
Primer sequences	Essential
RTPrimerDB Identification Number	Desirable
Probe sequences	Desirable ⁴
Location and identity of any modifications	Essential
Manufacturer of oligonucleotides	Desirable
Purification method	Desirable
qPCR Protocol	
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
qPCR Validation	
Evidence of optimization (from gradients)	Desirable
Specificity (gel, sequence, melt, or digest)	Essential
For SYBR Green I, C _q 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 ² of standard curve	Essential
Linear dynamic range	Essential
C _q 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
Data Analysis	
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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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.

³Assessing 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.

⁴Disclosure 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.