

Essential Items on the MIQE Checklist

Category	Item to be described/detailed	Location in Manuscript	Comments by Author
Sample	Type (blood, etc.)		
	Method of dissection/procurement		
	Processing procedure		
	If frozen, how and how quickly?		
	If fixed, with what and how quickly?		
	Storage conditions and duration		
Extraction	Method or instrument		
	Reagents/kits/modifications		
	DNase or RNase treatment		
	Evidence for lack of contamination (DNA or RNA)		
	Nucleic acid quantification		
	RNA integrity		
Reverse Transcription	Complete reaction conditions, including all components and their concentrations		
	RNA amount and reaction volume		
	Priming oligo sequence(s)		
	Cqs with and without reverse transcriptase		
qPCR target	HUGO gene abbreviation		
	Sequence accession number		
	Amplicon length		
	<i>In silico</i> specificity (BLAST)		
	Location by exon/intron		
	Identify the splice variants amplified		
	All primer/probe sequences		
	Location and identity of any oligonucleotide modifications		
qPCR protocol	Complete reaction conditions, including all components and their concentrations		
	cDNA/DNA amount and reaction volume		
	Instrument identification and complete thermocycling parameters		
qPCR validation	Evidence for PCR specificity (gels, sequencing, or melting curves)		
	Template inhibition data (template titrations)		
	For SYBR Green I reactions, the Cq of the no template control		

	Calibration curves with slope and intercept		
	PCR efficiency from the slope		
	r^2 of the calibration curve		
	Evidence for the linear dynamic range		
	Evidence for the limit of detection		
	For multiplexed assays, the efficiency and limit of detection of each assay		
Data analysis	qPCR analysis method/software		
	Method of Cq determination		
	Results of no template controls		
	Justification of number and choice of reference genes		
	Normalization method		
	Number and stage (reverse transcription or qPCR) of technical replicates		
	Intra-assay variation in terms of concentration, not Cq		
	Statistical methods/software		