**MIQE Checklist**

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| |  |  |  |  | | --- | --- | --- | --- | | **Category** | **Item to be described/detailed** | **Page No.** | **Author Comments** | | **SAMPLE** | Cell lines | 4 | Row 99 | |  | Method of dissection/procurement |  |  | |  | Processing procedure |  |  | |  | If frozen, how and how quickly? |  |  | |  | If fixed, with what and how quickly? |  |  | |  | Storage conditions and duration |  |  | | **EXTRACTION** | TRIzol reagent | 6 | Row 153 | |  | Reagents/kits/modifications | 6 | Total RNA was extracted according to the manufacturer’s protocol. The cDNA synthesis was performed using a SensiFAST cDNA synthesizer kit. | |  | DNAse or RNAse treatment | 6 | Total RNA was extracted according to the manufacturer’s protocol. The cDNA synthesis was performed using a SensiFAST cDNA synthesizer kit. | |  | Evidence for lack of contamination (DNA or RNA) | 6 | Total RNA was extracted according to the manufacturer’s protocol. The cDNA synthesis was performed using a SensiFAST cDNA synthesizer kit. | |  | Nucleic acid quantification | 6 | Total RNA was extracted according to the manufacturer’s protocol. The cDNA synthesis was performed using a SensiFAST cDNA synthesizer kit. | |  | RNA integrity | 6 | Total RNA was extracted according to the manufacturer’s protocol. The cDNA synthesis was performed using a SensiFAST cDNA synthesizer kit. | | **REVERSE TRANSCRIPTION** | SensiFAST cDNA synthesizer kit | 6 | Row 155 | |  | RNA amount and reaction volume |  | The cDNA synthesis was performed using a SensiFAST cDNA synthesizer kit. | |  | Priming oligo sequence(s) |  | The cDNA synthesis was performed using a SensiFAST cDNA synthesizer kit. | |  | Cqs with and without reverse transcriptase |  |  | | **qPCR TARGET** | HUGO gene abbreviation |  |  | |  | Sequence accession number |  |  | |  | Amplicon length |  |  | |  | *In silico* specificity (BLAST) | 16 | Primer sets of all glycosyltransferases are listed in Table 2 | |  | Location by exon/intron |  |  | |  | Identify the splice variants amplified |  |  | |  | All primer/probe sequences | 16 | listed in Table 2 | |  | Location and identity of any oligonucleotide modifications |  |  | | **qPCR PROTOCOL** | Complete reaction conditions, including all components and their concentrations | 6 | The gene amplification condition was performed as previously described (26)  Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett. 2020;20(3):2483-92. | |  | cDNA/DNA amount and reaction volume |  | The gene amplification condition was performed as previously described (26)  Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett. 2020;20(3):2483-92. | |  | Instrument identification and complete thermocycling parameters |  | The gene amplification condition was performed as previously described (26)  Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett. 2020;20(3):2483-92. | | **qPCR VALIDATION** | Evidence for PCR specificity (gels, sequencing, or melting curves) | 6 | The gene amplification condition was performed as previously described (26)  Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett. 2020;20(3):2483-92. | |  | Template inhibition data (template titrations) |  | The gene amplification condition was performed as previously described (26)  Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett. 2020;20(3):2483-92. | |  | For SYBR Green I reactions, the Cq of the no template control |  |  | |  | Calibration curves with slope and intercept |  | The gene amplification condition was performed as previously described (26)  Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett. 2020;20(3):2483-92. | |  | PCR efficiency from the slope |  | The gene amplification condition was performed as previously described (26)  Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett. 2020;20(3):2483-92. | |  | r2 of the calibration curve |  | The gene amplification condition was performed as previously described (26)  Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett. 2020;20(3):2483-92. | |  | 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 | 6 | Relative mRNA expression was determined using the 2Ct method (27).  Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8. | |  | Method of Cq determination |  |  | |  | Results of no template controls |  |  | |  | Justification of number and choice of reference genes |  |  | |  | Normalization method | 6 | *β*-*Actin* was used as the internal control to normalize the expression of the target genes. | |  | Number and stage (reverse transcription or qPCR) of technical replicates |  | Relative mRNA expression was determined using the 2Ct method (27).  Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8. | |  | Intra-assay variation in terms of concentration, not Cq |  |  | |  | Statistical methods/software | 7 | All analyses were performed with GraphPad Prism software (version 8.0; GraphPad Software, Inc.). A P < 0.05 indicated a statistically significant difference. | |