**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.  |

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