MQ Probe 2X qPCR Universal Mix

Cat. No. PQM-M-002-100
Size: 1ml (100 Reactions)
Store at -20°C under dark condition

MOLEQULE-ON®

Kit Components	Quantity
MQ Probe 2X qPCR Universal Mix	1 ml

Description

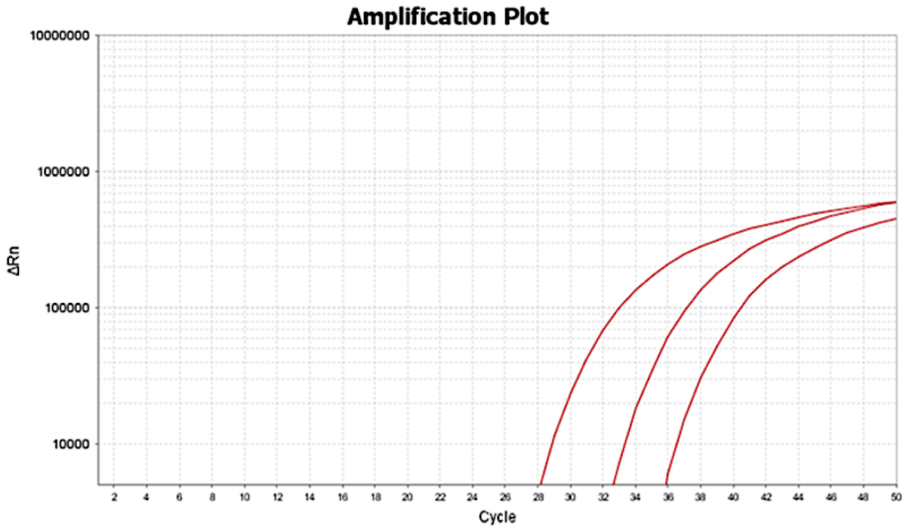
MQ Probe 2X qPCR Universal Mix is a 2X concentrated, ready-to-use master mix optimized for probe-based real-time PCR and compatible with the majority of commercially available real-time PCR systems (ROX-independent and ROX-dependent). It contains antibody-mediated hot-start Taq DNA polymerase, dNTPs, $MgCl_2$, enhancers, stabilizers and essentials for a success PCR reaction.

Applications

MQ Probe qPCR Mix is ideally suited for:
- Multiplex qPCR
- Gene expression analysis (absolute and relative)
- Detection of low copy genes
- Quantification of viral loads or NGS libraries

Primer Designing

Please verify the specificity of the primer pair by blasting the template's organism (Primer-BLAST: http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primers should amplify an amplicon with 80 – 200 bp. Do not exceed 400 bp. Extension and annealing time can reduced by amplification of smaller amplicons. Using the default settings of primer3 software, the melting temperature should be 60 °C.



DNA conc.	5000copy	500copy	50copy
Ct	26.652	31.640	35.133

Performance of MQ Probe 2X qPCR Universal Mix

The SARS-CoV-2 nucleocapsid (N1) gene with serial diluted DNA template concentrations are prepared and conducted by qPCR. Based on the detected N1 probe fluorescence values, the result shows the Ct values corresponding to the different concentration.

Procedure

Step 1: Prepare the PCR master mix

1. Thaw MQ Probe 2X qPCR Universal Mix and the rest of frozen reaction components to a temperature of 4°C. In order to entirely collect solutions, combine thoroughly and centrifuge briefly, then store at 4°C and avoid from light.
2. Prepare (on ice or at room temperature) enough assay Master Mix for all reactions by adding all necessary components, except the DNA template, according to the recommendations in Table 1 (below).

Components	20 µl reaction	Final Conc.
PCR-grade water	Up to 20 µl	N/A
2X MQ Probe Mix	10 µl	1X
Forward Primer	Variable	300-500mM
Reverse Primer	Variable	300-500nM
Probe	Variable	150-250 nM
Template DNA	Variable	cDNA: 1pg-10ng Genomic DNA: 50-250ng

Step 2: Set up individual reactions

1. Combine the assay Master Mix thoroughly to ensure consistency and equally dispense the solution into each qPCR tube or into the wells of a qPCR plate. Employ good pipetting practice to ensure assay precision and accuracy.
2. Add DNA samples (and DNase-free H_2O if needed) to the PCR tubes or wells containing assay Master Mix (Table 1), seal the tubes or wells with flat caps or optically transparent film. Note: to ensure thorough mixing of reaction components, vortex for approximately 30 seconds (or more).
3. Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.
4. Setup the thermal cycling protocol on a real-time PCR instrument according to Table 2. Note: optimization may be needed for better performance.
5. Load the PCR tubes or plate into the real-time PCR instrument and commence the run.
6. Perform data analysis according to the instrument-specific instructions.

Step 3: Run the PCR

Process in the thermal cycler for 35~45 cycles as follows:

Step	Temperature	Duration	Cycle
Initial denaturation	95 ºC	3-5 min[1]	1
Denaturation	95 ºC	15 Sec	40
Annealing[2] & Elongation	60 - 65 ºC	60 Sec	
Melt analysis	OPTIONAL		

1 Initial denaturation for 3 min at 95 °C is recommended for most assays. For GC-rich targets (>65% GC), 5 min at 95 °C may be used.

2 An annealing temperature 5 °C lower than the calculated melting temperature (Tm) of the primer set is recommended as a first approach.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantifying of nucleic acid targets with the kit.

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	Do not store diluted template in water or at low concentrations. Check the integrity of template material by automated or manual gel electrophoresis.
	Inadequate Thermal Cycling Conditions	Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. Use a solution of 10% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Primer Design	Verify primers design at different annealing temperatures.
Low or High Reaction Efficiency	Primer- Dimer	Reduce primer concentration. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary. Perform melt-curve analysis to determine if primer- dimers are present.
	Insufficient Optimization	Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.