MQ One Step Probe 2X qPCR Ready Mix

Cat. No. OSP-M-001-100
Size: 100 Reactions
Store at -20°C under dark condition

MOLEQULE-ON®

Kit Components	Quantity
MQ 2X Universal Probe qPCR Mix	1 ml
MQ RT Enzyme Mix	20 µl

Description

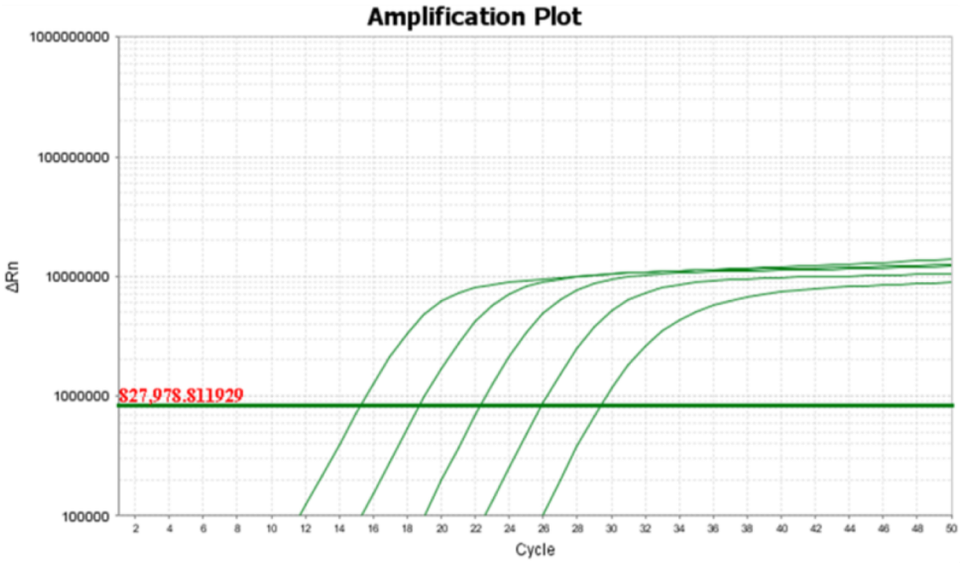
MQ OneStep probe qPCR Mix delivers high sensitivity of the target RNA level due to its unique reverse transcriptase, a reduced RNase H+ activity MMLV enzyme in addition to a powerful RNase inhibitors mix which aim to diminish RNA degradation and mispriming during reaction setup and reverse transcription to guarantee optimal RT efficiency.
The Universal qPCR Master 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 One Step Green qPCR Mix is ideally suited for:
- Gene expression analysis (absolute and relative)
- Detection of low copy genes

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.



RNA conc.	2pg (1.5*10^7 copy)	200fg (1.5*10^6 copy)	20fg (1.5*10^5 copy)	2fg (1.5*10^4 copy)	200ag (1.5*10^3 copy)
Ct	14.761	18.29	21.546	24.713	27.676

Performance of MQ One Step Probe 2X qPCR Ready Mix

To evaluate the performance, the Ribonuclease P (RP) gene with varying RNA template concentrations, through serial dilutions, were used. Based on the fluorescence value of the RP probe, the results show the performance corresponding to the Ct value with different copy numbers.

Procedure

1. Thaw MQ RT Enzyme Mix, MQ 2X Universal Probe 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 RNA template, according to the recommendations in Table below;

Components	20 µl reaction	Final Conc.
PCR-grade water	Up to 20 µl	N/A
MQ 2X Universal Probe Mix	10 µl	1X
MQ RT enzyme Mix	0.2 µl	1X
Forward Primer (10 µM)	Variable	300 nM
Reverse Primer (10 µM)	Variable	300 nM
Fluorogenic Probe (s)	Variable	150-250nM Each
Template RNA	Variable	1ng-5ug

3. 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.
4. Add RNA template (and DNase-free H_2O if needed) to the PCR tubes or wells containing assay Master Mix (According to Table), 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).
5. Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.
6. Setup the thermal cycling protocol on a real-time PCR instrument according to below Table. Note: optimization may be needed for better performance.
7. Load the PCR tubes or plate into the real-time PCR instrument and commence the run.
8. Perform data analysis according to the instrument-specific instructions.

Step	Temperature	Duration	Cycle
cDNA Synthesis	42 ºC	15 min	1
Pre-Denaturation	95 ºC	5 min	1
Denaturation	95 ºC	10 Sec	35-45
Annealing & Elongation	60 ºC	60 Sec	
Instrument Cooling	10 sec on 40 ºC		

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

Template:
Purified high quality RNA is needed for a success RT-qPCR reaction.

Important Notes:
1. Shake gently before use to avoid foaming and low-speed centrifugation.
2. During operation, always wear a lab coat, disposable gloves, and protective equipment.

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.