MQ One Step Probe qPCR Mix

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

MOLEQULE-ON®

Kit Components	Quantity
MQ One Step Probe Ready Mix	1 ml
MQ 20X Scriptase	100 µl

Description

Combining the reverse transcription with a qPCR detection mix results in the optimal convenience protocol therefore lowering the risk of contamination by avoiding additional pipetting. The reverse transcriptase implemented in this kit is a modified MuLV, engineered to fasten the process of turning the RNA to DNA. This enables a 10 min reverse trancsription rather than a 1 hour step using the wild type enzyme. Extensive research allowed us to create a buffer mixture optimal for the detection of multiple fluorophores within a mixture. This research also led to the development of buffer chemistries optimizied for earlier CT -values in a qPCR assay. Combining the earlier CT -values with the multiplex, the MQ One Step Probe qPCR Mix is perfect for qPCR assays using Probes.

Applications

MQ One Step Probe qPCR Mix is ideally suited for:
- Multiplex RT-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.

Procedure

Step 1: Prepare the PCR master mix

Ensure that all reagents are properly thawed and mixed.

1. Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of the reactions to be performed.
2. Calculate the required volumes of each component based on the following table:

Components	20 µl reaction	Final Conc.
PCR-grade water	Up to 20 µl	N/A
MQ One Step Probe Ready Mix	10 µl	1X
MQ 20X Scriptase	1 µl	1X
Forward Primer (10 µM)	0.8 µl	400 nM
Reverse Primer (10 µM)	0.8 µl	400 nM
Probe (10µM)	0.4 µl	200 nM
Template RNA	1 pg - 1 µg total RNA, >0.01 pg mRNA	As required

Step 2: Set up individual reactions

1. Transfer the appropriate volume of PCR master mix, template and primer to individual PCR tubes/wells or a PCR plate.
2. Cap or seal individual reactions, mix and centrifuge briefly.

Step 3: Run the PCR

Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycle
Reverse Transcription	45 ºC	10 min	1
Initial denaturation	95 ºC	2 min[1]	1
Denaturation	95 ºC	5 Sec	40
Annealing[2] & Elongation	60 - 65 ºC	20 – 30 Sec	
Melt analysis	OPTIONAL		

1 Initial denaturation for 2 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. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature of the primer pair.

Instrument compatibility

High ROX concentration (500 nM)	
Manufacturer	**Model**
ThermoFisher Scientific	7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™, StepOne™plus

Low ROX concentration (50 nM)	
Manufacturer	**Model**
Agilent	MX3000P, MX3005P, MX4000P
Analytik Jena	qTower
Bio-Rad	CFX96, CFX 384, Chromo4, MiniOpticon, Opticon, Opticon™ 2
Cepheid	SmartCycler
Eppendorf	Mastercycler ep realplex, Mastercycler ep realplex 2S
Fluidigm	BioMark
Hain Lifesciences	FluoroCycler®96
PCR$_{max}$	Eco™ 48
Qiagen	RotorGene™ 3000, RotorGene™ 6000, RotorGene™ Q
Roche	LightCycler® 480, LightCycler® 96, LightCycler® Nano
Takara	Thermal Cycler Dice®(TP800)
Techne	PrimeQ, Quantica
ThermoFisher Scientific	7500, 7500 FAST, Piko Real®, QuantStudio™12k Flex, ViiA7™