MQ Probe 2X qPCR Universal Mix

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

MOLEQULE-ON®

Kit Components	Quantity
MQ Probe 2X qPCR Universal Mix	1 ml
50 µM ROX Additive	200 µl

Description

Through extensive research, a buffer mixture has been created which is optimal for the detection of multiple fluorophores within a mixture. This research also led to the development of buffer chemistries optimized for earlier C_T-values in a qPCR assay. Combining the earlier C_T-values with the multiplex, the MQ Probe 2X qPCR Universal Mix is perfect for qPCR assays using Probes. This ready to use mix can replace any commercial Probe based qPCR mixture. The annealing temperature may need to be optimized to account for differences in buffer formulation. MQ Probe qPCR mix has be optimized for multiplex qPCR and it comes with separate ROX vial for addition to the master mix.

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.

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
2X MQ Probe	10 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 DNA	1 µg genomic DNA 100 ng cDNA	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
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.

Preparation of ROX

The passive reference dye ROX has been widely used for loading control and volume normalization. Many modern instruments do not need the ROX reference dye at all. The concentration of the reference dye is dependent on the instrument. Please check the instrument's manual for further information. The kit comes with a 50 µM ROX solution, which can be added according to the necessities of the instrument (500 nM for high ROX and 50 nM for low ROX concentration):

Reagent	High ROX	Low ROX
2X Mix	1 ml	1 ml
50 µM ROX Additive	20 µl	2 µl
ROX concentration in Reaction	500 nM	50 nM