

## Introduction

**LiQuant™ Probe qPCR Master Mix** is a ready-to-use solution optimized for TaqMan probe-based real time PCR analysis of DNA samples. The master mix includes HotStart Taq DNA Polymerase, MgCl<sub>2</sub>, dNTPs and other proprietary buffer components, which have been optimized to deliver superb sensitivity and performance in under one hour. HotStart Taq DNA Polymerase has 5'→3' polymerase activity and 5'→3' exonuclease activity, which is essential for TaqMan probe hydrolysis during qPCR. It does not have 3'→5' exonuclease (proofreading) activity, and produces 3'-dA-tailed amplicons, which are compatible with TA cloning if needed. TaqProbe qPCR Master Mix is suitable for a variety of applications, including SNP genotyping assays, gene expression analysis, microarray validation, and high-throughput screening.

## Package Information

Components	M0027
2× LiQuant™ Probe qPCR Mix*	4× 1.25ml
ROX Reference Dye**	100 µl

\* Contains dNTP Mix, Mg<sup>2+</sup>, and LiQuant™ DNA Polymerase. Keep all components, reaction mixes and samples on ice.

\*\* ROX Reference Dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is supplied at a 50 µM concentration. Use the following table to determine the amount of ROX to use with a particular instrument:

DO NOT USE ROX Reference Dye	BioRad: iCycler iQ, MyiQ, iQ5, CFX-96, CFX-384. Eppendorf: Mastercycler ep realplex. Roche: LightCycler 480, LightCycler 2.0.
USE ROX Reference Dye (20 µl)	Applied Biosystems: ABI 7000, 7300, 7700, 7900HT, StepOne, StepOne Plus
USE ROX Reference Dye (2 µl)	Applied Biosystems: QuantStudio 3, QuantStudio 5, QuantStudio 6 Flex, QuantStudio 7 Flex, 7500, 7500 Fast, ViiA7,. Stratagene: Mx3000P, Mx3005P, Mx4000

## Storage

Store at -20°C and protect from light.  
Aliquot after the first use to avoid repeated freeze-thaw cycles.

## Primer Designing Notes

1. The primer length should be 17 bp-25 bp.
2. GC content of the primers should be within the range of 40%-60% or 45%-55% as preferred.

## LiQuant™ Probe qPCR Master Mix

Cat. #: M0027 Size: 500 rxns

3. A, G, C, and T should be distributed as equally as within the primer. Avoid using GC- or TA-rich regions.
4. Avoid continuous T/C and A/G structures in primer design.
5. Avoid using > 2 G or C at the last 5 bases of the 3'-end of primer.
6. Both the forward and reverse primers should be close to the region of TaqMan Probe, but should not overlap with it.

## TaqMan Probe Designing Notes

1. The TaqMan Probe should be close to the regions of the forward and reverse primers, but should not overlap with them.
2. The length of TaqMan Probe should be 18 bp-40 bp.
3. Avoid continuous identical bases, especially GGGG or more.
4. At the 5'-ends, avoid using G.
5. The annealing temperature of probe should be 65°C-67°C.
6. If there is polymorphic locus in the sequence, make it lie in the middle of the probe.

## Protocol

Assemble qPCR reactions in a nuclease-free environment. Use of "clean" dedicated pipettes and aerosol resistant barrier tips are recommended.

1. Thaw template DNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Prepare the following reaction mixture in a qPCR tube on ice:

Component	Volume	Concentration
2× LiQuant™ Probe qPCR Mix	10 µl	1×
Forward Primer (10 µM)	0.5 µl	250 nM
Reverse Primer (10 µM)	0.5 µl	250 nM
TaqMan Probe (10 µM)	0.4 µl	250 nM
Template DNA/cDNA	x µl	1-100 ng
ddH <sub>2</sub> O	To 20 µl	-

**Note:** Check the instrument to determine the ROX amount to be added to qPCR Master Mix.

3. Mix carefully by vortexing for 3 -5 seconds. Centrifuge briefly to collect the contents of the tube.

4. Perform qPCR reaction using the recommended thermal cycling conditions outlined below:

Steps	Temperature	Duration	Cycle
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	15 sec	40
Annealing/Extension	60°C	60 sec	

**Note:**

1. Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
2. qPCR Master Mix component is light sensitive; avoid prolonged exposure to intense light.
3. Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to qPCR reaction.

**Troubleshooting**

**Abnormal shape of amplification plot**

1. Rough amplification plot: Caused by system rectification due to weak signal. Elevate the template concentration and repeat the reaction.
2. Broken or downward amplification plot: Concentration of templates is too high. The end value of the baseline is bigger than Ct value. Decrease the end of the baseline (Ct value - 4) and re-analyze the data.
3. Amplification plot goes downward suddenly: Bubbles left in the tube break up when the temperature rises, shown as sudden decrease of the fluorescence value. Spin briefly and check closely if there are bubbles left before PCR.

**No amplification plot**

1. Insufficient cycling: The cycling number is set to be 40. Cycling with too many cycles leads to excessive background and reduces the data reliability.
2. Signals are not read during cycling: In 2-step PCRs, read signals during annealing and extension. In 3-step PCRs, read signals during extension.
3. Primers are degraded: Test the integrity of primers (i.e. after long-term storage) using PAGE electrophoresis.
4. Low template concentration: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without dilution in template.
5. Degradation of templates: Prepare new templates and repeat the PCR.

**Ct value is too high**

1. Low amplification efficiency: Optimize the PCR system (i.e. try 3-step PCR or re-design the primers).

**LiQuant™ Probe qPCR Master Mix**

Cat. #: M0027 Size: 500 rxns

2. Low template concentration: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without dilution in template.
3. Degradation of templates: Prepare new templates and repeat the PCR.
4. The amplicon is too long: The recommended amplicon size is within 100 bp-150 bp.
5. PCR inhibitors in the system: Usually brought in when adding templates. Increase the dilution folds or prepare new templates, and then retry.

**Amplification observed in negative control**

1. Contaminated reagents or water: Use new reagents or water and retry. Prepare the reaction system in a clean bench.
2. Primer dimers: It's normal to observe amplification of primer dimers in negative control after 35 cycles, which can be identified in the melt curve.

**Poor fitness of the standard curve using linear regression in absolute qPCR**

1. Deviations of pipetting volume: Dilute the templates and increase the pipetting volume accordingly.
2. Degradation of standards: Prepare new standards and retry.
3. High template concentration: Increase the dilution fold.

**Poor reproducibility**

1. Inaccurate pipetting volume: Use a more accurate pipettor, increase the pipetting volume by increasing the reaction volume and diluting the templates.
2. Difference in temperature control between wells in qPCR instrument: Maintain the instruments periodically.
3. Low template concentration: The lower the template concentration, the worse the reproducibility. Decrease the dilution fold or increase the volume.