August 2023

Quick-Start Protocol

qPCR Probe Mix

The qPCR Probe Mix (cat. no. AM04-020 and AM04-200) is an enzyme mixture for quantitative Real-Time PCR using probes, including TaqMan®, Scorpions® and molecular beacon probes. It is used for probe-based Real-Time PCR assays, including singleplex, and multiplex gene expression studies, genotyping experiments.

Ready-to-use, 2x concentrated Master Mix contains TaqNova HS DNA Polymerase which is a mixture of recombinant Taq polymerase over-expressed in *E. coli* and a specific monoclonal anti-Taq antibody. The TaqNova HS DNA Polymerase enables set-up of a hot-start PCR reaction at room temperature. The antibody binds reversibly to the enzyme, inhibiting polymerase activity at ambient temperatures, which prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during PCR set-up. The antibody is released from the polymerase during initial DNA denaturation step, thus providing full DNA polymerase activity during standard cycling conditions.
The qPCR Probe Mix ships on dry ice. Storage in the long term is at –20°C and after thawing at 2–8°C for two months. It does not lose its activity after eight successive freeze/thaw cycles.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Procedure

1. Prior to use, thaw the reagents completely, mix thoroughly by pipetting or vortexing and spin briefly. Avoid direct light during next steps.
2. Prepare the qPCR Master Mix by combining the following reaction reagents in a sterile nuclease-free tube:

Table 1. Real-Time PCR reaction mixture content

Reagent	Suggested amount per reaction	Acceptable final concentrations in reaction mixture
2x qPCR Probe Mix	10 µL	1x
Forward primer (10 µM)	0.6 µL (0.3 µM)	0.1 – 0.8 µM
Reverse primer (10 µM)	0.6 µL (0.3 µM)	0.1 – 0.8 µM
Probe (10 µM)*	0.2 µL (0.1 µM)	0.05 – 0.4 µM
DNA or cDNA template	1 – 100 ng	1 pg – 0.5 µg
PCR-grade water	fill up to 20 µL	

* For best results the final probe concentration should be 2-3x lower than the primer concentration. In multiplex Real-Time PCR probe concentrations over 0.1 µM can result in cross-channel fluorescence.

3. Determine the total volume for the appropriate number of reactions, plus 10% overage and prepare the Master Mix of all reagents except DNA template. Mix the components by pipetting or inverting the tube and spin briefly.
4. Aliquot the contents into qPCR tubes or multiple wells of qPCR reaction plate.
5. Add DNA templates to qPCR tubes/plate.
6. Seal the plate with qPCR foil or cap qPCR tubes with optical caps.
7. Spin qPCR tubes/plate for 1–2 min to remove air bubbles and collect liquid to the bottom of the tube.
8. Transfer qPCR tubes/plate to a thermal cycler block and run qPCR reaction.
9. Program your qPCR instrument with the following conditions:

   9a. If possible, select FAST cycling option.

9b. Choose the detection channel of the qPCR instrument that corresponds with the fluorophore used in the assay.

9c. Set a thermal cycling profile according to the tables below (note that the following conditions are suitable for amplicons of up to 250 bp and may vary depending on different instrument-specific protocols).

Table 2. Three-step thermal cycling profile

Step	Temperature(°C)	Time(s)	Cycle
Activation and denaturation	95	180	
Denaturation	95	5	35 – 45 cycles
Annealing	60	10	
Extension / Fluorescence Detection	72	5 – 20	

Table 3. Two-step thermal cycling profile

Step	Temperature(°C)	Time(s)	Cycle
Activation and denaturation	95	180	
Denaturation	95	5	35 – 45 cycles
Annealing / Extension / Fluorescence detection	60	15 – 30	

* It is not recommended to use annealing/extension times longer than 30 seconds.

Document Revision History

Date	Changes
08/2023	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.