August 2023

Quick-Start Protocol

One-Step Probe RT-qPCR Mix

The One-Step Probe RT-qPCR Mix (cat. nos. AM08.1-100 and AM08.1-500) is a reaction mixture for RT-qPCR using probes including TaqMan®, Scorpions®, and molecular beacon probes. It was created for reproducible and efficient first-strand cDNA synthesis and subsequent real-time PCR in a single tube.

The qPCR MIX should be stored at –20°C. Its components do not lose their activity after eight successive freeze–thaw cycles, and aliquoting can be applied if necessary.

Further information

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

Things to do before starting

1. Thaw the reagents completely and mix thoroughly by pipetting or inverting the tube and spin briefly. Avoid direct light during next steps.
2. Prepare the RT-qPCR Master Mix placed on ice or in a freezing rack by combining the following reaction reagents in a sterile nuclease-free tube according to Table 1.

Table 1. Suggested RT-qPCR reaction mixture content

Reagent	Amount	Final concentration
One-Step Probe RT-qPCR Mix	10 µL	1x
Forward primer (10 µM)	0.8 µL (0.4 µM)	0.2–1 µM
Reverse primer (10 µM)	0.8 µL (0.4 µM)	0.2–1 µM
Probe (10 µM)	0.2 µL (0.1 µM)	0.05–0.4 µM
RNase Inhibitor Hu	0.1 µL	–
Reverse Transcriptase	0.1 µL	–
RNA template	up to 8 µL	1 pg – 1 µg (Total RNA) from 0.01 pg (mRNA)
Nuclease-free water	up to 20 µL	–

Procedure

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

   7a. If possible, select FAST cycling option.

   7b. Choose the detection channel of the qPCR instrument that corresponds with the fluorophore used in the assay.

   7c. Set a thermal cycling profile according to Table 2.

   Note: the following conditions are suitable for amplicons of up to 200 bp and may vary depending on different instrument-specific protocols).

Table 2. Two-step thermal cycling profile

Step	Temperature (°C)	Time (s)	Cycle
Reverse transcription*	45	900	–
Activation and denaturation	95	180	–
Denaturation	95	5	40
Annealing / Extension / Fluorescence Detection†	60	45	40

* The reverse transcription reaction time can be between 10–20 min and temperature can be increased up to 50°C.

† The annealing/extension time can be between 30–60 s and temperature can be increased up to 65°C.

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.