Quick-Start Protocol October 2015

QIAGEN® OneStep Ahead RT-PCR Kit

The QIAGEN OneStep Ahead RT-PCR Kit (cat. nos. 220211, 220213 and 220216) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. The OneStep Ahead RT-PCR Master Mix can also be stored at 2–8°C for up to 6 months, depending on the expiration date.

Further information

- *QIAGEN OneStep Ahead RT-PCR Kit Handbook*: **www.qiagen.com/HB-1998**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Reverse transcription and PCR are carried out sequentially in the same tube. All components required for both reactions are added during setup, and there is no need to add additional components once the reaction has been started.
- The protocol has been optimized for 0.1 pg – 1 µg of total RNA.
- The blend of DNA polymerases, including a proofreading enzyme, contained in the QIAGEN OneStep Ahead RT-PCR Master Mix, requires a heat-activation step of 5 min at 95°C after the reverse transcription step. This also inactivates the reverse transcriptases.
- The OneStep Ahead RT Mix contains an RNase inhibitor and a blend of Omniscript® and Sensiscript® reverse transcriptases in combination with an RT-blocker for heat-mediated activation of the reverse-transcription step.
- Heat-mediated activation of the reverse transcriptases prevents nonspecific enzyme activity. This allows reaction setup at room temperature, facilitating the use of this kit in automated (robotic) workflows.

- The kit is designed for use with gene-specific primers at a final concentration of 0.5 µM. The use of random oligomers or oligo-dT primers is not recommended.
- QIAGEN OneStep Ahead RT-PCR Buffer provides a final concentration of 2.5 mM $MgCl_2$ in the reaction mix, which provides satisfactory results in most cases.
- The OneStep Ahead Template Tracer and the OneStep Ahead Master Mix Tracer contain blue and orange dyes, respectively, that allow tracking of pipetted samples during RT-PCR setup. The use of these tracers is optional. When the blue template is added to the orange OneStep Ahead RT-PCR Master Mix, the color changes to green. The Template Tracer is provided as a 25x concentrate and should be used in a 1x final concentration in the sample. To generate a template dilution series, dilute the 25x concentrate (using template and water) to obtain a final concentration of 1x OneStep Ahead Blue Template Tracer in the diluent. The Master Mix Tracer is provided as a 125x concentrate and can be added directly to the Master Mix stock vial to obtain a 1x concentration in the final reaction mix. These tracers do not reduce sample stability or RT-PCR performance.
- Reactions can be directly loaded onto an agarose gel after cycling. Tracer dyes allow monitoring of the loading process and tracking during subsequent electrophoresis. The dyes run at approximately 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.
- The QIAGEN OneStep Ahead RT-PCR Kit contains Q-Solution®, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution.

1. Thaw OneStep Ahead RT-PCR Master Mix, template RNA, primer solutions, RNase-free water and 5x Q-Solution (optional). Mix thoroughly before use.
2. Prepare a reaction mix according to Table 1. The reaction mix contains all the components except the template RNA. Prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.

   Due to the 2-phase hot-start of both the RT and the PCR reactions, it is not necessary to keep samples on ice during reaction setup or while programming the cycler.

   Note: A negative control (without template RNA) should be included in every experiment.

Table 1. Reaction setup for one-step RT-PCR

Component	Volume/reaction	Final concentration
Reaction mix OneStep Ahead RT-PCR Master Mix, 2.5x	10 µl	1x
OneStep Ahead RT-Mix, 25x	1 µl	1x
Primer A	Variable	0.5 µM
Primer B	Variable	0.5 µM
RNase-Free Water	Variable	–
Optional: OneStep Ahead Master Mix Tracer,125x	0.2 µl	1x
Optional: 5x Q-Solution*	5 µl	1x
Template RNA (added at step 4)	Variable	0.1 pg – 1 µg/reaction
Total reaction volume	**25 µl**	

* For templates with GC-rich regions or complex secondary structure.

3. Mix the reaction mixture gently but thoroughly, for example, by pipetting up and down a few times or vortexing a few seconds. Dispense appropriate volumes into PCR tubes or wells of a PCR plate.
4. Add template RNA (1 µg – 100 fg per reaction, depending on target transcript abundance) to the individual PCR tubes. The QIAGEN OneStep Ahead RT-PCR Kit can be used with total RNA, messenger RNA, viral RNA or in vitro transcribed RNA.
5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Tables 2 and 3. The protocol includes steps for both reverse transcription and PCR.
6. Place the PCR tubes or plates in the thermal cycler and start the RT-PCR program.
   Note: After amplification, store samples at –20°C for long-term storage.
7. We have evaluated several specialized protocols and particular hints. For details, please refer to the *QIAGEN OneStep Ahead RT-PCR Kit Handbook*, which can be found at **www.qiagen.com/HB-1998**.

Table 2. One-step RT-PCR cycling conditions for amplicons < 1 kbp

Step	Time	Temperature	Comment
Reverse transcription	10 min	50°C	OmniScript and SensiScript RTs are activated and reverse transcription takes place. If satisfactory results are not obtained at 50°C, increase the temperature up to 55°C.
Initial PCR activation	5 min	95°C	This activates the DNA Polymerase blend, inactivates Omniscript and Sensiscript Reverse Transcriptases and denatures the cDNA template.
3-step cycling: Denaturation	10 s	95°C	Do not exceed this temperature.
Annealing	10 s*	55°C	Approximately 5°C below T_m of primers.
Extension	10 s*	72°C	For RT-PCR products up to 1 kb, an extension time of 10 s is sufficient.
Number of cycles	40		The optimal cycle number depends on the amount of template RNA and the abundance of the target transcript.
Final extension	2 min	72°C	

* For duplex RT-PCR, increase the time to 20 s.

Table 3. One-step RT-PCR cycling conditions for amplicons 1-4 kbp

Step	Time	Temperature	Comment
Reverse transcription	15 min	45°C	OmniScript and SensiScript RTs are activated and reverse transcription takes place.
Initial PCR activation	5 min	95°C	This activates the DNA Polymerase blend, inactivates Omniscript and Sensiscript Reverse Transcriptases, and denatures the cDNA template.
3-step cycling: Denaturation	15 s	95°C	Do not exceed this temperature.
Annealing	15 s	55°C	Approximately 5°C below T_m of primers.
Extension	1–4 min	68°C	Allow 1 min per kbp amplicon size.
Number of cycles	40		The optimal cycle number depends on the amount of template RNA and the abundance of the target transcript.
Final extension	5 min	72°C	

Scan QR code for handbook.