Quick-Start Protocol March 2016

QIAGEN® OneStep RT-PCR Kit

The QIAGEN OneStep RT-PCR Kit (cat. nos. 210210, 210212 and 210215) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- *QIAGEN OneStep RT-PCR Handbook*: **www.qiagen.com/HB-0454**
- 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 1 pg – 2 µg of total RNA.
- HotStarTaq® DNA Polymerase, contained in the QIAGEN OneStep RT-PCR Enzyme Mix, requires a heat-activation step of 15 min at 95°C. This also inactivates the reverse transcriptases. Do not heat-activate the HotStarTaq DNA Polymerase until the reverse-transcriptase reaction is finished.
- QIAGEN OneStep RT-PCR Kits are designed to be used with gene-specific primers at a final concentration of 0.6 µM. The use of random oligomers or oligo-dT primers is not recommended.
- Set up all reactions on ice.
- Preheat the thermal cycler to 50°C before placing samples in it.
- QIAGEN OneStep RT-PCR Buffer provides a final concentration of 2.5 mM $MgCl_2$ in the reaction mix, which will give satisfactory results in most cases.

- QIAGEN OneStep RT-PCR Kits are provided with 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 template RNA, primer solutions, dNTP Mix, 5x QIAGEN OneStep RT-PCR Buffer, RNase-free water and 5x Q-Solution (optional) and place on ice. 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.

   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		
QIAGEN OneStep RT-PCR Buffer, 5x	10 µl	1x; 2.5 mM Mg^{2+}
dNTP mix (10 mM each)	2 µl	400 µM of each dNTP
Primer A	Variable	0.6 µM
Primer B	Variable	0.6 µM
RNase-free water	Variable	–
QIAGEN OneStep RT-PCR Enzyme Mix	2 µl	
Optional: 5x Q-Solution*	10 µl	1x
Optional: RNAse Inhibitor (not provided)	Variable	5–10 units/reaction
Template RNA (added at step 4)	Variable	1 pg – 2 µg/reaction
Total reaction volume	50 µl	

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

3. Mix the reaction mix gently but thoroughly, for example, by pipetting up and down a few times. Dispense appropriate volumes into PCR tubes.
4. Add template RNA (≤2 µg/reaction) to the individual PCR tubes. The QIAGEN OneStep RT-PCR Kit can be used with total RNA, messenger RNA or viral RNA.
5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 2. The protocol includes steps for both reverse transcription and PCR and gives satisfactory results in most cases. For maximum yield and specificity, temperatures and cycling times should be further optimized for each new target and primer pair.

Table 2. Cycling conditions for one-step RT-PCR

Step	Time	Temperature	Comment
Reverse transcription	30 min	50°C	The reaction temperature may be increased up to 60°C if satisfactory results are not obtained at 50°C.
Initial PCR activation	15 min	95°C	This heating step activates HotStarTaq DNA Polymerase, inactivates Omniscript® and Sensiscript® Reverse Transcriptases and denatures the cDNA template.
3-step cycling:			
Denaturation	0.5–1 min	94°C	Do not exceed this temperature.
Annealing	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers.
Extension	1 min	72°C	For RT-PCR products of 1–2 kb, increase the extension time by 30–60 s.
Number of cycles	25–40		The optimal cycle number depends on the amount of template RNA and the abundance of the target transcript.
Final extension	10 min	72°C	

6. Start the RT-PCR program while PCR tubes are still on ice. Place the PCR tubes in the thermal cycler once it has reached 50°C.

   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

7. We have evaluated several specialized protocols and particular hints for the cases listed in Table 3. For details, please refer to the indicated appendix in the *QIAGEN OneStep RT-PCR Handbook*, which can be found at **www.qiagen.com/HB-0454**.

Table 3. Specialized protocols

Specialized protocol	Appendix in the *QIAGEN OneStep RT-PCR Handbook*
Use of degenerate RT-PCR primers	B
Determination of cycle number based on target abundance	C
Increase of sensitivity by nested PCR	D
Long RT-PCR fragments >2 kb	F
Multiplex RT-PCR	G
Co-amplification of an internal control	H

Scan QR code for handbook.

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