QIAGEN Supplementary Protocol

Using the QIAGEN® OneStep RT-PCR Kit and Q-Solution® with a 25 µl reaction volume and shorter reaction times for viral samples

This protocol is designed for using Q-Solution in one-step RT-PCR **with a 25 µl reaction volume and shorter reaction times**. The protocol has been optimized for use with viral samples. The shortened reaction times enable time savings of 25%. Q-Solution changes the melting behavior of nucleic acids and can be used for RT-PCR systems that do not work well under standard conditions. When using Q-Solution the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution. This recommendation should also be followed if another RT-PCR additive (such as DMSO) was previously used for a particular primer–template system.

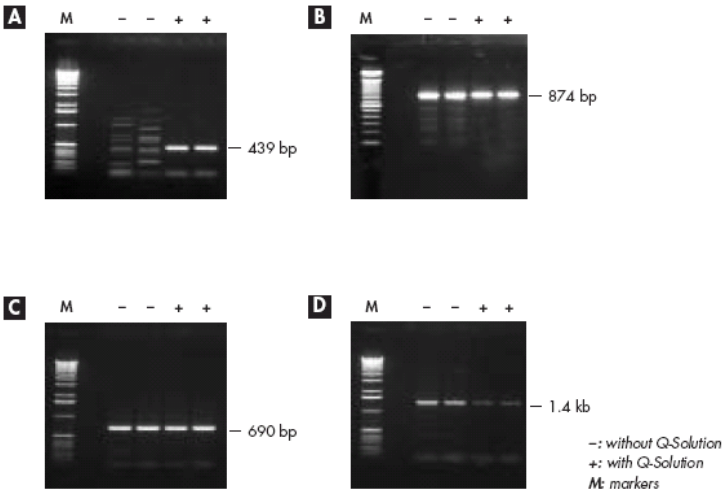
When using Q-Solution, the following effects may be observed depending on the individual RT-PCR assay:

Case A: Q-Solution enables RT-PCR that previously failed.

Case B: Q-Solution increases RT-PCR specificity in certain primer–template systems.

Case C: Q-Solution has no effect on RT-PCR performance.

Case D: Q-Solution causes reduced efficiency or failure of a previously successful amplification reaction. In this case, addition of Q-Solution disturbs the previously optimal primer–template annealing. Therefore, when using Q-Solution for the first time for a particular primer–template system, always perform reactions in parallel with and without Q-Solution.



IMPORTANT: Please consult the "Safety Information" section in the *QIAGEN OneStep RT-PCR Kit Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- QIAGEN OneStep RT-PCR Kit (cat. no. 210210 or 210212)
- Primers: The QIAGEN OneStep RT-PCR Kit is designed to be used with gene-specific primers. The use of random oligomers or oligo-dT primers is not recommended.
- RNase inhibitor (optional): RNase inhibitor is a 50 kDa protein that strongly inhibits RNases A, B, and C, as well as human placental RNases. It helps to minimize the risk of RNA degradation during experimental setup. The use of RNase inhibitor is optional because the buffer composition has an inhibitory effect on RNases.

Important points before starting

- In most cases this protocol will provide satisfactory results in a shorter amount of time. However, if increased sensitivity is desired, the optimized protocol listed in the *QIAGEN OneStep RT-PCR Kit Handbook* should be followed.
- HotStarTaq® DNA Polymerase, contained in the QIAGEN OneStep RT-PCR Enzyme Mix, requires **initial activation by incubation at 95°C for 15 min** before amplification can take place (see step 6 of this protocol). This incubation also inactivates the reverse transcriptases. Do not heat activate the HotStarTaq DNA Polymerase until the reverse-transcriptase reaction is finished.
- Q-Solution modifies the melting behavior of nucleic acids and can be used for primer–template systems that do not perform well using standard conditions. When using Q-Solution for the first time for a particular primer–template system, always perform parallel reactions with and without Q-Solution.
- The QIAGEN OneStep RT-PCR Kit is designed for use with **gene-specific primers** at a final concentration of **0.6 µM**. The use of random oligomers or oligo-dT primers is not recommended since it will result in the amplification of nonspecific products.
- Set up all reactions on ice.
- Make sure the thermal cycler is preheated to 50°C before placing samples in it.
- The 5x QIAGEN OneStep RT-PCR Buffer provides a final concentration of 2.5 mM $MgCl_2$ in the reaction mix, which will produce satisfactory results in most cases.
- An RNase-free environment should be maintained during RNA isolation and reaction setup.
- Set up reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

1. **Thaw viral RNA samples, primer solutions, dNTP Mix, 5x QIAGEN OneStep RT-PCR Buffer, Q-Solution, and RNase-free water, and place them on ice.**

   It is important to mix the solutions completely before use to avoid localized differences in salt concentration.

2. **Prepare a master mix according to Table 1.**

   The master mix typically contains all the components required for RT-PCR except the template RNA. Prepare a volume of master mix 10% greater than that required for the total number of reactions to be performed. A negative control (without template RNA) should be included in every experiment (see Appendix J in the *QIAGEN OneStep RT-PCR Kit Handbook*).

   When using Q-Solution for the first time for a particular primer–template system, always perform parallel reactions with and without Q-Solution.

3. **Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.**

   Mix gently, for example, by pipetting the master mix up and down a few times.

4. **Add viral RNA to the individual PCR tubes.**

   The QIAGEN OneStep RT-PCR Kit can be used with total RNA, messenger RNA, or viral RNA. However, this protocol is optimized for use with viral RNA samples. The volume of template RNA should not exceed 40% of the total reaction volume.

5. **Program the thermal cycler according to the program outlined in Table 2.**

   Table 2 describes a typical thermal cycler program. The program includes steps for both reverse transcription and PCR. The PCR amplification segment must start with an initial heating step at 95°C for 15 min to activate HotStarTaq DNA Polymerase. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target and primer pair.

6. **Start the RT-PCR program while PCR tubes are still on ice. Wait until the thermal cycler has reached 50°C. Then place the PCR tubes in the thermal cycler.**

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

Table 1. Reaction components for one-step RT-PCR using Q-Solution

Component	Volume/reaction	Final concentration
Master mix		
RNase-free water (provided)	Variable	–
5x QIAGEN OneStep RT-PCR Buffer*	5.0 µl	1x
dNTP Mix (containing 10 mM of each dNTP)	1.0 µl	400 µM of each dNTP
5x Q-Solution	5.0 µl	1x
Primer A	Variable	**0.6 µM**†
Primer B	Variable	**0.6 µM**†
QIAGEN OneStep RT-PCR Enzyme Mix	1.0 µl	–
RNase inhibitor (optional)‡	Variable	5–10 units/reaction
Template RNA		
Viral RNA sample, added at step 4	Variable	Up to a maximum of 40% of final reaction volume
Total volume	**25.0 µl**	**–**

* Contains 12.5 mM $MgCl_2$

† A final primer concentration of 0.6 µM is optimal for most primer–template systems. However, in some cases using other primer concentrations (i.e., 0.5–1.0 µM) may improve amplification performance.

‡ The use of RNase inhibitor is optional because the buffer composition has an inhibitory effect on RNAses.

Table 2. Thermal cycler conditions

			Additional comments
Reverse transcription:	20 min	50°C	A reverse-transcription reaction temperature of 50°C is recommended.
Initial PCR activation step:	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step. Omniscript® and Sensiscript® Reverse Transcriptases are inactivated and the cDNA template is denatured.
3-step cycling			
Denaturation:	30 s	95°C	
Annealing:	32 s	50–68°C	Approximately 5°C below T_m of primers.
Extension:	40 s	72°C	For RT-PCR products up to 300 bp. For longer fragments of up to 1.5 kb, increase the extension time by 5 s per 100 bp.
Number of cycles:	25–40		The cycle number is dependent on the amount of template RNA and the abundance of the target transcript. See Appendix C in the *QIAGEN OneStep RT-PCR Kit Handbook*.
Final extension:	2 min	72°C	

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

The QIAGEN OneStep RT-PCR Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

www.qiagen.com

Australia ■ 1-800-243-800
Austria ■ 0800/281010
Belgium ■ 0800-79612
Brazil ■ 0800-557779
Canada ■ 800-572-9613
China ■ 0086-21-3865-3865
Denmark ■ 80-885945
Finland ■ 0800-914416
France ■ 01-60-920-930
Germany ■ 02103-29-12000
Hong Kong ■ 800 933 965
Ireland ■ 1800 555 049
Italy ■ 800-787980
Japan ■ 03-6890-7300
Korea (South) ■ 1544 7145
Luxembourg ■ 8002 2076
Mexico ■ 01-800-7742-639
The Netherlands ■ 0800 0229592
Norway ■ 800-18859
Singapore ■ 65-67775366
Spain ■ 91-630-7050
Sweden ■ 020-790282
Switzerland ■ 055-254-22-11
UK ■ 01293-422-911
USA ■ 800-426-8157

Sample & Assay Technologies