QIAGEN Supplementary Protocol

QIAGEN® OneStep RT-PCR Kit Research Protocol for swine-origin influenza A virus (S-OIV) using end-point PCR

This protocol is for use in swine-origin influenza A (H1N1) virus (S-OIV) research applications using standard gene-specific primers. When using degenerate primers, please refer to the World Health Organization (WHO) protocol recommendations (www.who.int/csr/resources/publications/swineflu/WHO_Diagnostic_RecommendationsH1N1_20090521.pdf). 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. Optimal reaction conditions, such as incubation times and temperatures during PCR amplification, will vary and need to be determined individually.

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.

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.

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

- 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.
- The QIAGEN OneStep RT-PCR Kit is 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 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 the 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, 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*).

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.

5. **When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50 µl mineral oil.**

6. **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 can be further optimized for each new target and primer pair. However, the protocol gives satisfactory results in most cases.

7. **Start the RT-PCR program while PCR tubes are still on ice.**

   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

Component	Volume/reaction	Final concentration
Master mix		
RNase-free water (provided)	Variable	–
5x QIAGEN OneStep RT-PCR Buffer*	5 µl	1x
dNTP Mix (containing 10 mM of each dNTP)	1 µl	400 µM of each dNTP
Primer A	Variable	**0.6 µM**†
Primer B	Variable	**0.6 µM**†
QIAGEN OneStep RT-PCR Enzyme Mix	1 µl	–
RNase inhibitor (optional)‡	Variable	5–10 units/reaction
Template RNA		
Template RNA, added at step 4	5 µl	Up to a maximum of 40% of final reaction volume
Total volume	25 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 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

Step	Time	Temperature	Additional comments
Reverse transcription	30 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	0.5–1 min	94°C	
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. For RT-PCR products over 2 kb, see Appendix F in the *QIAGEN OneStep RT-PCR Kit Handbook*.
Number of cycles	35–45		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	10 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.

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