# QIAGEN Supplementary Protocol

## QIAGEN® OneStep RT-PCR Kit Research Protocol for swine-origin influenza A (H1N1) virus (S-OIV)

This protocol is for use in S-OIV research applications using primer and probe sequences available from the World Health Organization (WHO) (www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/). Reverse transcription and probe-based real-time 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 use with viral samples. Reaction volumes and times have also been shortened to enable time savings of 25%.

**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.
- TaqMan® probes: These should be purchased from an established oligonucleotide manufacturer and probe stock solutions should be protected from exposure to light.
- 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.

- Applied Biosystems® 7500 or other real-time PCR cycler
- PCR tubes or plates (use thin-walled PCR tubes or plates recommended by the manufacturer of the cycler)
- **Optional**: Trizma® base and EDTA for preparing TE buffer for storing primers and probes. Use RNase/DNase-free water and plastic consumables to prepare TE buffer.
- ROX dye might be required as a passive reference dye on certain real-time cyclers; please follow the instructions provided by the manufacturer of your cycler to determine if ROX dye has to be added in the reaction.

## 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 5 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.
- Set up all reactions on ice.
- 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.
- 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. 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 real-time cycler according to the program outlined in Table 2.**

   Table 2 describes a typical real-time 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.

6. **Place the PCR tubes in the cycler and start the RT-PCR program.**

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

7. **Perform data analysis.**

   Before performing data analysis, select the analysis settings (i.e., baseline settings and threshold values). Note that optimal analysis settings are a prerequisite for accurate quantification data.

**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 |
| Probe | Variable | 0.2 μM |
| Primer A | Variable | 0.8 μM |
| Primer B | Variable | 0.8 μM |
| QIAGEN OneStep RT-PCR Enzyme Mix | 1 μl | – |
| **Template RNA** | | |
| Viral RNA sample, added at step 4 | 5 μl | – |
| Total volume | 25 μl | – |

* Contains 2.5 mM $MgCl_2$

**Table 2. Cycling conditions**

| | | | 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. |
| 2-step cycling | | | |
| Denaturation: | 15 s | 95°C | |
| Annealing/Extension: | 60 s | 55°C* | Combined annealing/extension step with fluorescence data collection. |
| Number of cycles: | 45 | | |

* When using standard (not degenerate) primers and probes, please use an annealing/extension temperature of 60°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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