QIAGEN Supplementary Protocol

QuantiTect® Virus Kit research protocol for S-OIV

This protocol is for use in swine-origin influenza A (H1N1) virus (S-OIV) research applications using sequences available from the World Health Organization (WHO) (www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/). Real-time one-step RT-PCR is performed on the Applied Biosystems® 7500 or other real-time PCR cycler using a QuantiTect Virus Kit in combination with degenerate primers and probe.

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the *QuantiTect Virus 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.

QuantiTect Virus Kits are 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.

- **For all real-time PCR cyclers from Applied Biosystems except the Applied Biosystems 7500**: QuantiTect Virus Kit (cat. no. 211011, 211013, or 211015)
- **For the Applied Biosystems 7500 and real-time PCR cyclers from other suppliers**: QuantiTect Virus +ROX Vial Kit (cat. no. 211031, 211033, or 211035)
- Primers and probes: These should be purchased from an established oligonucleotide manufacturer. Primers should be of standard quality, and probes should be HPLC purified. Lyophilized primers and probes should be dissolved in TE buffer to provide a stock solution of 100 μM; concentration should be checked by spectrophotometry (for details, see Appendix A in the *QuantiTect Virus Handbook*). Primer and probe stock solutions should be stored in aliquots at –20°C. Probe stock solutions should be protected from exposure to light.
- Nuclease-free (RNase/DNase free) consumables: Special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive detection of viral nucleic acids. See Appendix C in the *QuantiTect Virus Handbook* for details about avoiding nucleases during PCR setup.
- Cooling device or ice

- 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 (see Appendix A in the *QuantiTect Virus Handbook*). Use RNase/DNase-free water and plastic consumables to prepare TE buffer.

Important points before starting

- **Always start with the cycling conditions specified in this protocol.**
- **Use the primer concentrations specified in this protocol.**
- **After reverse transcription, the PCR must start with an initial incubation step of 5 min at 95°C to activate HotStarTaq® *Plus* DNA Polymerase.**
- **Optimal analysis settings are a prerequisite for accurate quantification data.** For data analysis, you should always readjust the analysis settings (i.e., baseline settings and threshold values) for analysis of every reporter dye channel in every run.

Procedure

1. **Thaw 5x QuantiTect Master Mix, 50x ROX Dye Solution (if supplied), primer and probe solutions, RNase-free water, template nucleic acids (isolated viral nucleic acids), optional standards, and references. Mix the individual solutions.**

   Standards should be diluted in QuantiTect Nucleic Acid Dilution Buffer at an appropriate concentration to enable use of 2.5–5 µl per reaction.

2. **Prepare a reaction mix for the required number of reactions according to Table 1. It is recommended to prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.**

   Typically, reaction setup can be done at room temperature (15–25°C). However, it is recommended to keep the reagents, samples, and controls on ice or in a cooling device.

   Note: QuantiTect Virus RT Mix should be taken from –15 to –30°C immediately before use, always kept on ice, and returned to –15 to –30°C immediately after use.

3. **Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.**
4. **Add template nucleic acids to the individual PCR tubes or wells and mix thoroughly.**

   Note: Ensure that the reaction mix and template are thoroughly mixed.

5. **Program the real-time cycler according to Table 2.**

Table 1. Reaction setup

Component	Volume per 25 μl reaction	Final concentration
Reaction mix		
5x QuantiTect Master Mix*	5 μl	1x
50x ROX Dye Solution†	0.5 μl	1x
Forward primer	Variable	0.8 μM‡
Reverse primer	Variable	0.8 μM‡
Probe	Variable	0.2 μM§
100x QuantiTect Virus RT Mix	0.25 μl	1x
RNase-free water	Variable	–
Template RNA (added at step 4)	5 μl	–
Total volume per reaction	**25 μl**	**–**

* **For users of Applied Biosystems cyclers except the Applied Biosystems 7500**: use the 5x QuantiTect Virus Master Mix supplied with the QuantiTect Virus Kit. **For users of the Applied Biosystems 7500 and cyclers from other suppliers**: use the 5x QuantiTect Virus NR Master Mix supplied with the QuantiTect Virus +ROX Vial Kit.

† The ROX dye solution is required for the Applied Biosystems 7500 and is optional for cyclers from Stratagene. For all other cyclers, use water instead.

‡ A final primer concentration of 0.8 μM is optimal. Before adapting primer concentration, verify the concentration of your primer solutions.

§ A final probe concentration of 0.2 μM gives satisfactory results when using the InfA, SW InfA, and RNase P primer–probe sets. **For the SW H1 primer–probe set, use a final probe concentration of 0.4 μM.**

Table 2. Cycling conditions

Step	Time	Temperature	Additional comments
Reverse transcription	**20 min**	**50°C**	RNA is reverse transcribed into cDNA.
Initial PCR activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this heating step.
2-step cycling:			**Important: Optimal performance is only assured using these cycling conditions.**
Denaturation	**15 s**	**95°C**	
Annealing/extension	**45 s**	**55°C**	Combined annealing/extension step with fluorescence data collection.
Number of cycles	45		

6. **Place the PCR tubes or plate in the real-time cycler and start the PCR cycling program.**
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.

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.

Trademarks: QIAGEN®, HotStarTaq®, QuantiTect® (QIAGEN Group); Applied Biosystems® (Applera Corporation or its subsidiaries); Trizma® (Sigma-Aldrich Co.). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

NOTICE TO PURCHASER: LIMITED LICENSE
A license to perform the 5' nuclease process for research requires the use of a Licensed 5' Nuclease Kit (containing Licensed Probe), or the combination of an Authorized 5' Nuclease Core Kit plus Licensed Probe, or license rights that may be purchased from Applied Biosystems. This product (QuantiTect Virus Kits) is an Authorized 5' Nuclease Core Kit without Licensed Probe. Its purchase price includes a limited, non-transferable immunity from suit under U.S. Patents Nos. 5,210,015, 5,487,972, 5,476,774, and 5,219,727, and corresponding patent claims outside the United States, owned by Roche Molecular Systems, Inc. or F. Hoffmann-La Roche Ltd (Roche), for using only this amount of the product in the practice of the 5' nuclease process solely for the purchaser’s own internal research when used in conjunction with Licensed Probe. This product is also an Authorized 5' Nuclease Core Kit for use with service sublicenses available from Applied Biosystems. This product conveys no rights under U.S. Patents Nos. 5,804,375, 6,214,979, 5,538,848, 5,723,591, 5,876,930, 6,030,787, or 6,258,569, or corresponding patents outside the United States, expressly, by implication or by estoppel. No right under any other patent claims (such as apparatus or system claims in U.S. Patent No. 6,814,934) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

NOTICE TO PURCHASER: LIMITED LICENSE
The purchase price of this product (QuantiTect Virus Kits) includes a limited, non-transferable license under U.S. Patents Nos. 5,407,800, 5,322,770, 5,310,652 and corresponding patent claims outside the United States, owned by Roche Molecular Systems, Inc. or F. Hoffmann-La Roche Ltd (Roche), to use only this amount of product solely for the purchaser’s own internal research. No right under any other patent claims (such as apparatus or system claims) and no right to use this product for any other purpose or for commercial services of any kind, including without limitation reporting the results of purchaser’s activities for a fee or other commercial consideration, is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

PCR110 Aug-09 © 2009 QIAGEN, all rights reserved.

www.qiagen.com
Australia ■ 1-800-243-800
Austria ■ 0800/281010
Belgium ■ 0800-79612
Canada ■ 800-572-9613
China ■ 021 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 ■ 03-5547-0811
Korea (South) ■ 1544 7145
Luxembourg ■ 8002 2076
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