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virotype® Influenza A RT-PCR Kit Handbook

Σ 24 (catalog no. 282603)

Σ 96 (catalog no. 282605)

Σ 480 (catalog no. 282607)*

For detection of RNA from influenza A virus

Registered in accordance with § 17c of the German Law on Animal Diseases (FLI-B 538)

REF 282603, 282605, 282607*

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* Available only on request.

Contents

Kit Contents

virotype **Influenza A RT-PCR Kit**	**(24)**	**(96)**	**(480)**
Catalog no.	**282603**	**282605**	**282607***
Number of reactions	**24**	**96**	**480**
Master Mix (tube with orange cap) includes enzymes, primers, and probes	1 x 500 µl	2 x 980 µl	6 x 1625 µl
Positive Control (tube with red cap)	1 x 25 µl	1 x 70 µl	2 x 50 µl
Negative Control (tube with blue cap)	1 x 25 µl	1 x 70 µl	2 x 50 µl
Handbook	1	1	1

* Available only on request.

Intended Use

The *virotype* Influenza A RT-PCR Kit is intended for the detection of RNA from influenza A virus in oropharyngeal, tracheal, and cloacal swabs (individual or pooled), fecal samples, or tissue samples from birds; nasal swabs, bronchoalveolar lavage fluid (BALF), and tissue samples from swine, as well as nasal swabs from equids. The kit is approved by the Friedrich-Loeffler-Institut and registered in accordance with § 17c of the German Law on

Animal Diseases (FLI-B 538) for use in Germany for veterinary diagnostic procedures. **For veterinary use only.**

Symbols

 <N>	Contains reagents for <N> plates
	Legal manufacturer
	Lot number
	Use by date
	Temperature limitations for storage
	Consult instructions for use
	Catalog number
	Material number
	Protect from light
	For samples from birds, swine and equids

Storage

The components of the *virotype* Influenza A RT-PCR Kit should be stored at –30 to –15°C and are stable until the expiration date stated on the label. Avoid repeated thawing and freezing (>2x), as this may reduce assay sensitivity. Freeze the components in aliquots if they will only be used intermittently.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *virotype* Influenza RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *virotype* Influenza A RT-PCR Kit is a highly sensitive solution for the safe and sensitive detection of RNA from influenza A virus in samples from birds, pigs and equids. Viruses of the genus *Influenzavirus* A belong to the family *Orthomyxoviridae*. They occur in high genetic diversity and a wide range of virulence. Influenza A viruses are grouped into low and highly pathogenic strains. Waterfowl are the natural reservoir of low-pathogenic avian influenza viruses (LPAIV). Highly pathogenic avian influenza viruses (HPAIV) belong to subtypes H5 or H7 and may cause fowl plague in domestic poultry with high economic losses. The subtypes H1N1, H1N2, and H3N2 of influenza A virus can also cause infections of the respiratory tract in swine as can the subtype H3N8 in equids.

Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time RT-PCR, the amplified product is detected using fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows the detection of the accumulating product without the need to re-open the reaction tubes afterward.

The *virotype* Influenza A RT-PCR Kit contains all of the necessary reagents for the detection of influenza A RNA, including a

positive and negative control. With this kit, both reverse transcription and PCR are performed in one reaction tube, reducing the risk of contamination.

The kit uses two specific primer/probe combinations: one for influenza A RNA yielding FAM™ fluorescence and one for a housekeeping gene (β-actin mRNA), present within the sample, yielding HEX™ fluorescence.

A Positive Control serves to verify the functionality of the reaction mix for the amplification of the influenza A RNA target.

RNA extraction

The *virotype* Influenza A RT-PCR Kit can be used for the detection of influenza A RNA from the following sample types:

- birds: oropharyngeal, tracheal, and cloacal swabs (individual or pooled), fecal samples, tissue samples
- swine: nasal swabs, bronchoalveolar lavage fluid (BALF), tissue samples
- equids: nasal swabs

Due to the high sensitivity of the test, pools of up to 10 individual swab samples can be analyzed.

Prior to real-time RT-PCR, viral RNA must be extracted from the starting material. QIAGEN offers a range of products for RNA extraction from animal samples.

- QIAamp® *cador*® Pathogen Mini Kit
- QIAamp Viral RNA Mini Kit
- RNeasy® Fibrous Tissue Mini Kit

If real-time RT-PCR is not performed immediately after extraction, store the RNA at –20°C or at –70°C for longer storage.

RNA extraction using kits based on spin-column technology can be automated using the QIAcube®.

Equipment and Reagents to Be Supplied by User

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

Equipment

- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Rotor-Gene® Q or 96-well plate real-time cycler with appropriate fluorescent channels
- Rotor-Gene Q software version 1.7.94 or higher, or appropriate software for chosen 96-well plate cycler

Material

- Pipettes
- Nuclease-free, aerosol-resistant pipette tips with filters
- Sterile 1.5 ml Eppendorf® tubes
- 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 identification of viral nucleic acids.
- Strip Tubes and Caps, 0.1 ml, for use with Rotor-Gene Q (cat. no. 981103 or 981106) or 96-well optical microplate with

optical sealing film or cover for chosen 96-well plate real-time cycler

Important Notes

General precautions

The user should always pay attention to the following:

- Use nuclease-free pipette tips with filters.
- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components on ice before starting an assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test kit past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

Negative Control

At least one negative control reaction should be included in each PCR run. This enables assessment of contamination in the reaction.

Positive Control

When performing PCR on unknown samples, it is recommended to perform a positive control reaction in the PCR run, containing a sample that is known to include the targeted viral RNA. A positive control serves to prove the functionality of the pathogen assay, for example, the correct setup of the reaction mix. Use 5 µl of the Positive Control provided with the *virotype* Influenza A RT-PCR Kit to test for successful amplification of the target.

Extraction and amplification control

For increased process safety and convenience, an extraction and amplification control assay is included in the form of a second primer/probe set that detects a housekeeping gene present within the sample. This allows both extraction and amplification to be monitored.

Protocol: Real-time RT-PCR for Identification of Influenza A Virus

Important points before starting

- Please read “Important Notes” on page 10 before starting.
- Include at least one positive control (Positive Control) and one negative control (Negative Control) per PCR run.
- Before beginning the procedure, read through the protocol and ensure that you are familiar with the operation of the chosen real-time PCR cycler.
- RNA is unstable. Perform the protocol without interruption.

Things to do before starting

- Thaw all reagents on ice and protect from light.
- Maintain reagents on ice during PCR setup.
- Before use, centrifuge the reagents briefly.

Procedure

1. Pipet 20 µl of the Master Mix into each reaction tube. Then add 5 µl of the sample RNA (Table 1).

   Include positive and negative control reactions.

   Positive control: Use 5 µl of the positive control (Positive Control) instead of sample RNA.

   Negative control: Use 5 µl of the negative control (Negative Control) instead of sample RNA.

Table 1. Preparation of reaction mix

Component	Volume
Master Mix	20 µl
Sample	5 µl
Total volume	**25 µl**

2. Close the reaction tubes with the corresponding caps.
3. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2. Select the green and yellow channels on the Rotor-Gene Q.

Table 2. Filter setting for reporter

Pathogen/internal control	Reporter
Influenza A	FAM
Internal control	HEX/JOE™*
Passive reference†	ROX™

* Use the option appropriate for your thermal cycler.

† Internal reference for use with the Applied Biosystems® ABI PRISM® Sequence Detection Systems.

4. Run the real-time RT-PCR protocol according to Table 3 if running only the *virotype* Influenza A RT-PCR Kit.

Table 3. Real-time RT-PCR protocol for Influenza A

Temperature	Time	Number of cycles
45°C	10 min	1
95°C	10 min	1
95°C	15 s	40
60°C*	60 s	

* Fluorescence data collection.

5. Run the real-time RT-PCR protocol according to Table 4 if running other *virotype* assays simultaneously (i.e., *virotype* BTVpan/8, *virotype* BVDV, *virotype* CSFV, *virotype* PRRSV and/or *virotype* SBV).

Table 4. Real-time RT-PCR protocol for simultaneous assays

Temperature	Time	Number of cycles
50°C	20 min	1
95°C	15 min	1
95°C	30 s	40
57°C†	45 s	
68°C	45 s	

† Fluorescence data collection.

Data Analysis and Interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in both the FAM and HEX channels with a C_T* <35. The following results are possible if working with unknown samples.

The possible sample results are also summarized in Table 5 on page 17.

The sample is positive for influenza A virus, and the assay is valid, if the following criteria are met:

- The sample yields a signal in both the FAM and HEX† channels
- The Positive Control yields a signal in both the FAM and HEX channels
- The Negative Control does not yield a signal in the FAM and HEX channels

Note that very high concentrations of influenza A RNA in the sample may lead to a reduced HEX signal or no HEX signal due to competition with the internal control.

* Threshold cycle (C_T) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence.

† Green and yellow on the Rotor-Gene Q.

The sample is negative for influenza A virus, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the HEX channel but not in the FAM channel
- The Positive Control yields a signal in both the FAM and HEX channels
- The Negative Control does not yield a signal in the FAM and HEX channels

A positive HEX signal means that extraction and amplification were successful as the housekeeping gene (β-actin mRNA) within the sample is amplified.

The sample results are inconclusive, and the assay is invalid, if the following occurs:

- The sample yields no signal in the FAM and HEX channel

If no signal is detected in both the FAM (pathogen) and the HEX (Internal Control) channel, the result is inconclusive. The absence of a signal for the housekeeping gene indicates PCR inhibition and/or other malfunctions.

To check for inhibition, we recommend 1:5 dilution of the sample RNA in nuclease free water.

Check that there is a fluorescence signal in the FAM channel for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be

due to incorrect setup of the reaction mix or incorrect cycling conditions.

Table 5. Results interpretation table*

Sample result	Reporter	
	FAM (pathogen)	HEX (IC)
Influenza A positive	X	X
Influenza A positive (strong positive)	X	
Influenza A negative		X
Inconclusive result		

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The positive control should yield a signal in both the FAM and HEX channels. The negative control must yield no signal in the FAM and HEX channels. For a complete explanation of possible sample results please refer to "Data Analysis and Interpretation" on page 15.

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
virotype Influenza A RT-PCR Kit (24)	For 24 reactions: Master Mix, Positive Control, Negative Control	282603
virotype Influenza A RT-PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	282605
virotype Influenza A RT-PCR Kit (480)*	For 480 reactions: Master Mix, Positive Control, Negative Control	282607
Related products		
flocktype® AIV Ab (2)	For 196 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274012
bactotype® Mycoplasma Mg/Ms PCR Kit (96)†	For 96 reactions: Master Mix, Positive Control, Negative Control	288105
bactotype MAP PCR Kit (96)†	For 96 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285905
cador T. equigenitalis PCR Kit (24)	For 24 reactions: Master Mix, Positive Control, Internal Control, Mg-Sol, Water (PCR Grade)	285023

* Available only on request.

† Other kit sizes are available; see **www.qiagen.com**.

virotype ASFV PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281905
virotype BTV pan/4 RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	280455
virotype BTV pan/8 RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	280445
virotype BVDV RT-PCR Kit (96)*	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280375
virotype CSFV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281805
virotype PRRSV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282305
virotype SBV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281605
QIAamp *cador* Pathogen Mini Kit (50)*	For 50 preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers	54104
QIAamp Viral RNA Mini Kit (50)*	For 50 RNA preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-free Buffers	52904
RNeasy Fibrous Tissue Mini Kit (50)	For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers	74704

* Other kit sizes are available; see **www.qiagen.com**.

cador Pathogen 96 QIAcube HT Kit (5)	For 480 preps: QIAamp 96 plates, QIAGEN Proteinase Q, Carrier RNA, buffers	54161
MagAttract® 96 *cador* Pathogen Kit (384)	For 384 preps: 4 Large 96-Rod Covers, 24 S-Blocks, MagAttract Suspension G, Buffers and Reagents	947457
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor	9001570

QIAGEN offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit **www.qiagen.com/animal-health** for more information about *bactotype*, *cador*®, *cattletype*®, *flocktype*, *pigtype*® and *virotype* products.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Limited License Agreement for *virotype* Influenza A RT-PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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