June 2016

virotype® BTV pan/4 RT-PCR Kit Handbook

24 (catalog no. 280453)

96 (catalog no. 280455)

For detection of RNA from bluetongue virus (BTV) and BTV serotype 4

Licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-C 020)

REF 280453, 280455

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Contents

Kit Contents

***virotype* BTV pan/4 RT-PCR Kit**	**(24)**	**(96)**
Catalog no.	**280453**	**280455**
Number of reactions	**24**	**96**
Master Mix (tube with orange cap), includes enzymes, primers and probes	1 x 500 µl	2 x 980 µl
Positive Control (tube with red cap)	1 x 25 µl	1 x 70 µl
Negative Control (tube with blue cap)	1 x 25 µl	1 x 70 µl
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Intended Use

The *virotype* BTV pan/4 RT-PCR Kit is intended for the detection of bluetongue virus RNA in ruminant whole blood (individual and pooled samples) and tissue samples (spleen, lymph nodes) from cattle, sheep and goats.

The kit is approved by the Friedrich-Loeffler-Institut and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-C 020) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

 <N>	Contains reagents for <N> tests
	Legal manufacturer
	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
	Catalog number
	Material number
	Protect from light
	For cattle, sheep and goat samples

Storage

The components of the *virotype* BTV pan/4 RT-PCR Kit should be stored at –30°C 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 which have come into contact with samples must be decontaminated or disposed of as potentially infective material.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *virotype* BTV pan/4 RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

Bluetongue is an infectious, non-contagious disease of ruminants. The agent is the bluetongue virus (BTV), a double-stranded RNA virus of the genus *Orbivirus* of the family *Reoviridae* which includes at least 27 known serotypes. BTV is widely distributed around the world. Sheep, cattle and goats are mainly affected by the disease. Clear clinical signs are usually seen only in sheep. In severe cases the tongue may show intense hyperemia and become cyanotic (Bluetongue). BTV serotype 4 is of epidemiological importance in Europe and cause of recent Bluetongue Disease outbreaks. The virus is transmitted by certain midges of the genus *Culicoides*. Furthermore, the virus can be spread by contaminated needles and surgery equipment.

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 detection of the accumulating product without the need to re-open the reaction tubes afterward.

The *virotype* BTV pan/4 RT-PCR Kit contains all of the necessary reagents for the detection of BTV 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 *virotype* BTV pan/4 RT-PCR Kit uses three specific primer/probe combinations: one for the RNA of at least 27 known BTV serotypes yielding FAM™ fluorescence, one for BTV-4 RNA yielding Cy5™ fluorescence, and one for a housekeeping gene (β-actin mRNA), present within the sample, yielding HEX™ fluorescence.

The Positive Control contains BTV-4 in vitro RNA yielding Cy5 fluorescence and double-stranded BTV-8 RNA yielding FAM fluorescence. The detection of the FAM fluorescence allows the control of the denaturation step since the successful denaturation of the viral double-stranded RNA is a prerequisite for amplification.

RNA extraction

The *virotype* BTV pan/4 RT-PCR Kit can be used for the detection of BTV RNA from ruminant whole blood (preferred with anticoagulants, for example EDTA-blood) and tissue samples (spleen, lymph nodes). Due to the high sensitivity of the test, individual or pooled samples can be tested. Pools of up to 10 individual blood samples may be analyzed. However, the optimum pool size depends on the regional prevalence for the BTV.

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
- MagAttract® 96 cador Pathogen Kit
- QIAamp Viral RNA Mini Kit
- RNeasy® Fibrous Tissue Mini Kit for tissue
- RNeasy 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.

- Pipets
- Nuclease-free, aerosol-resistant pipet 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.
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Cooling device or ice
- 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
- 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 pipet 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* BTV pan/4 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 an additional 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 Using the Rotor-Gene Q

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, spin the reagents briefly.

Procedure

1. Pipet at least 7 µl of RNA samples or Positive Control into individual 0.2 ml PCR reaction tubes. Cover the reaction tubes (e.g., with PCR sealing film).

   Include positive and negative control reactions.

   Positive Control: Use at least 7 µl of the positive control (Positive Control) instead of sample RNA.
2. Denature the samples for 5 min at 98°C in a 96-well plate standard cycler with a heated lid.

3. Immediately cool down on ice water or liquid nitrogen for at least 20 s. Then store on ice or cooling device.
4. Pipet 5 µl of RNA samples, Positive Control, and Negative Control into individual Strip Tubes and Caps, 0.1 ml, for use with Rotor-Gene Q. Use 5 µl of the negative control (Negative Control) instead of sample RNA.
5. Add 20 µl of the Master Mix into each reaction tube. Thus the final volume is 25 µl (Table 1).

Table 1. Preparation of the reaction mix

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

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

Table 2. Filter settings for the reporter

Pathogen/Internal Control	Reporter
BTV pan	**FAM**
BTV-4	**Cy5**
Internal Control	HEX/JOE™*

* Use the option appropriate for your thermal cycler.

8. Run the real-time RT-PCR protocol according to Table 3 if running only the *virotype* BTV pan/4 RT-PCR Kit.

Table 3. Real-time PCR protocol for BTV pan/4

Temperature	Time	Number of cycles
50°C	10 min	1
95°C	10 min	1
95°C	15 s	40
60°C†	60 s	

† Fluorescence data collection.

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

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	
57°C*	45 s	40
68°C	45 s	

* Fluorescence data collection.

Protocol: Real-time RT PCR Using 96-Well Plate Real-Time Cycler

Please read “Important Notes”, page 10, and “Important points before starting” and “Things to do before starting”, page 12.

Procedure

1. Pipet 5 µl of RNA sample, Positive Control and Negative Control into individual reaction tubes. Cover the reaction tubes (e.g., with PCR sealing film).

   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.
2. Denature the samples for 5 min at 98°C in a 96-well plate standard cycler with a heated lid.
3. Immediately cool down on ice water or liquid nitrogen for at least 20 s. Then store on ice or in a cooling device.
4. Pipet 20 µl of the Master Mix into each reaction tube. Thus the final volume of a test is 25 µl (Table 5).

Table 5. Preparation of the reaction mix

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

5. Close the reaction tubes with the corresponding caps.
6. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 6.

Table 6. Filter settings for the reporter

Pathogen/Internal Control	Reporter
BTV pan	**FAM**
BTV-4	**Cy5**
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.

7. Run the real-time RT-PCR protocol according to Table 7 if running only the *virotype* BTV pan/4 RT-PCR Kit.

Table 7. Real-time RT-PCR Protocol for BTV pan/4

Temperature	Time	Number of cycles
50°C	10 min	1
95°C	10 min	1
95°C	15 s	40
60°C*	60 s	

* Fluorescence data collection.

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

Table 8. 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 the FAM, Cy5 and HEX channels with a C_T*<35. If no signal or a C_T ≥35 in the FAM channel of the Positive Control is measured, the denaturation of the double-stranded RNA and cooling steps were insufficient and the testing should be repeated. The Negative Control must give no signal.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 9 on page 21.

The sample is positive for BTV and BTV-4, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the FAM, Cy5 and HEX† channels.
- The Positive Control yields a signal in all channels.
- The Negative Control yields no signal.

Note that high concentrations of BTV 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 positive for BTV, negative for BTV-4, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the FAM and HEX channels but not in the Cy5 channel.
- The Positive Control yields a signal in all channels.
- The Negative Control yields no signal.

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

The sample is negative for both BTV and BTV-4, and the assay is valid, if the following criteria are met:

- The sample yields a signal only in the HEX channel.
- The Positive Control yields a signal in all channels.
- The Negative Control yields no signal.

A positive HEX signal rules out the possibility of PCR inhibition and/or incorrect RNA extraction as the internal control is amplified.

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

- The sample yields no signal in any of the fluorescence channels.

The PCR was inhibited or the sample extraction was incorrect. It is recommended to retest the respective individual samples in

nuclease free water (e.g., diluted 1:5), to repeat the RNA extraction, or to repeat the whole test procedure starting with new sample material.

Check that there is a fluorescence signal in all the channels for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be due to incorrect RNA denaturation or incorrect cycling conditions.

Repeat RNA extraction or repeat the whole procedure starting with new sample material.

Table 9. Results interpretation table*

	Reporter		
Sample result	**FAM**	**Cy5**	**HEX**
BTV positive	X		(X)
BTV-4 positive	X	X	(X)
Negative			X
Inconclusive result			

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The positive control must yield a signal in the FAM, Cy5 and HEX channels. The negative control must yield no signal. For a complete explanation of possible sample results, please refer to "Data Analysis and Interpretation" on page 19.

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 BTV pan/4 RT-PCR Kit (24)	For 24 reactions: Master Mix, Positive Control, Negative Control	280453
virotype BTV pan/4 RT-PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	280455
Related products		
virotype BTV pan/8 RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	280445
virotype SBV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281605
virotype BVDV RT-PCR Kit (96)*	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280375
bactotype MAP PCR Kit (96)*	For 96 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285905
virotype ASFV PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281905
virotype CSFV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281805
virotype PEDV/TGEV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	283605
virotype PRRSV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282305

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

Product	Contents	Cat. no.
virotype Influenza A RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282605
bactotype® Mycoplasma Mg/Ms PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	288105
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
DNeasy® Blood & Tissue Kit (50)*	For 50 preps: 50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504
QIAamp DNA Blood Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
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

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

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-and-Veterinary-Testing** 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* BTV pan/4 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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1100454 06/2016