November 2014

virotype® BVDV RT-PCR Kit Handbook

24 (catalog no. 280373)

96 (catalog no. 280375)

480 (catalog no. 280377)

For detection of RNA from bovine viral diarrhea virus

Registered in accordance with § 17c of the German Law on Animal Diseases (FLI-B 451)

280373, 280375, 280377

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Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

In addition, QIAGEN provides high-quality, easy-to-use, and sensitive molecular solutions to enable veterinary pathogen detection and animal pathogen research. The QIAGEN veterinary portfolio includes a broad range of pathogen-specific PCR-assays and an extensive and growing ELISA portfolio. For more information, visit www.qiagen.com/Animal-and-Veterinary-Testing.

Contents

Kit Contents

***virotype* BVDV RT-PCR Kit**	**(24)**	**(96)**	**(480)**
Catalog no.	**280373**	**280375**	**280377**
Number of reactions	**24**	**96**	**480**
PCR Mix (tube with yellow cap) includes primers, probes, and internal control	1 x 500 µl	2 x 1000 µl	6 x 1650 µl
Enzyme Mix (tube with green cap)	1 x 6.5 µl	1 x 26 µl	2 x 65 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	1 x 100 µl
Handbook	1	1	1

Intended Use

The *virotype* BVDV RT-PCR Kit is intended for the detection of bovine viral diarrhea virus RNA in blood, plasma, serum, milk and ear tissue samples (individual or pooled) from cattle. The kit is approved by the Friedrich-Loeffler-Institut and registered in accordance with § 17c of the German Law on Animal Diseases (FLI-B 451) for use in Germany for veterinary diagnostic procedures. For veterinary use only.

Symbols

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 samples

Storage

The components of the *virotype* BVDV RT-PCR Kit should be stored at –15 to –30°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* BVDV RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

Bovine Viral Diarrhoea (BVD) and Mucosal Disease (MD) are caused by Bovine Viral Diarrhoea Virus (BVDV I, II), a single-stranded RNA virus. It belongs to the genus pestivirus, as are Classical Swine Fever Virus and Border Disease Virus in sheep.

BVD is an economically important infectious disease of cattle. Depending on the immune status of the animals, BVDV infections may lead to gastro-intestinal and respiratory symptoms of different severity as well as to reproductive problems. These are caused by transplacental infection of the fetus leading to abortions, congenital malformations, and in case of infection before immunocompetence to persistently infected (PI or viraemic) calves. PI animals only emerge through prenatal infection, whereas postnatal infections lead to transient viraemia, inducing the production of neutralizing antibodies.

Unidentified PI animals are responsible for the spread of BVDV as they excrete high doses of the virus during their whole life. Thus they may infect pregnant animals which in turn may give birth to new PI animals. The major route to successfully combat the disease is the early identification of those PI animals.

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* BVDV RT-PCR Kit contains all of the necessary reagents for the detection of BVDV 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.
An internal control excludes the possibility of false negative results. The kit uses two specific primer/probe combinations: one for pestiviruses yielding FAM™ fluorescence and one for the internal control yielding HEX™ fluorescence.

RNA extraction

The *virotype* BVDV RT-PCR Kit is a highly sensitive solution for the detection of bovine viral diarrhea virus RNA in blood, plasma, serum, milk and ear tissue samples from cattle.

Preferably use EDTA plasma because viral RNA is minimally degraded in the presence of EDTA. Due to the high sensitivity of the test, pools consisting of 50 individual blood samples (blood, plasma, serum), pools of 100 individual milk samples and pooled ear tissue samples consisting of 25 individual samples

may be analyzed. However, the optimal pool size depends on the regional prevalence for BVDV and on the age of the animals.
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.

For rapid preparation of ear tissue samples (diameter 2–3 mm) without RNA isolation QIAGEN recommends *virotype* Tissue Lysis Reagent. Ear tissue lysates should be stored at –20 °C or at 2–8°C for up to 12 h.

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
- 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. Note that the Internal Control RNA will be detected in the negative control as it is included in the reaction mix as an amplification control (see "Internal Control" below).

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, e.g., the correct setup of the reaction mix. Use 5 µl of the

Positive Control provided with the *virotype* BVDV RT-PCR Kit to test for successful amplification of the target.

Internal Control

For convenience, the Internal Control RNA is already contained in the reagents provided. This eliminates the need to add the Internal Control to each sample separately during reaction setup. The Internal Control allows the user to monitor PCR inhibition.

Protocol: Real-time RT-PCR for identification of bovine viral diarrhea virus

Important points before starting

- Please read "Important Notes" on page 11 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.
- Remove the Enzyme Mix from storage at –20°C immediately before use. Keep it on ice. Return it to –20°C immediately after use.
- Before use, spin the reagents briefly

Procedure

1. **Prepare the master mix according to Table 1.**
   The master mix contains all of the components needed for PCR except the sample. Prepare a volume of master mix at least 10% greater than that required for the total number of PCR assays to be performed.
   See Table 1 for the volumes per number of reactions for the master mix.

Table 1. Preparation of master mix

Component	1	24	96	480
PCR Mix (yellow cap)	19.75 µl	493.75 µl	1896 µl	9480 µl
Enzyme Mix (green cap)	0.25 µl	6.25 µl	24 µl	120 µl
Total volume	20 µl	500 µl	1920 µl	9600 µl

2. **Pipet 20 µl of the master mix into each reaction tube. Then add 5 µl of the sample RNA (Table 2).**
   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 2. Preparation of reaction mix

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

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

Table 3. Filter settings for reporter and quencher

Component	Reporter	Quencher
BVDV/unknown sample	FAM	TAMRA™
Internal control	HEX/JOE™*	TAMRA
Passive reference†	ROX™	–

* Use the option appropriate for your thermal cycler.

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

5. Run the real-time RT-PCR protocol according to Table 4.

Table 4. Real-time RT-PCR protocol

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.

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* <36. The Negative Control must not give a signal in the FAM channel.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 5 on page 18.

The sample is positive for BVDV, 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 yields a signal in the HEX channel with a C_T <36
- The Negative Control does not yield a signal in the FAM channel

Note that very high concentrations of BVDV RNA in the sample may lead to a reduced HEX signal or no HEX signal due to competition with the internal control. If the C_T value of the FAM fluorescence is less than 30, the sample is most likely from a persistently infected animal (PI animal).

* 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.

Sample is negative for BVDV, 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 yields a signal in the HEX channel
- The Negative Control does not yield a signal in the FAM channel

A positive HEX signal means that PCR was successful 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 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 Internal Control 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 master mix or incorrect cycling conditions.

Table 5. Results interpretation table*

Sample result	Reporter FAM (pathogen)	HEX (IC)
BVDV positive	X	X
BVDV positive (strong positive)	X	
BVDV 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 both the FAM and HEX channels. The negative control must yield a signal in the HEX channel. For a complete explanation of possible sample results please refer to "Data Analysis and Interpretation" on page 16.

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, see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
virotype BVDV RT-PCR Kit (24)	For 24 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280373
virotype BVDV RT-PCR Kit (96)	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280375
virotype BVDV RT-PCR Kit (480)	For 480 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280377
Related products		
bactotype MAP PCR Kit (24)*	For 24 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285903
virotype TLR (25 ml)	25 ml Tissue Lysis Reagent	289991
virotype TLR (100 ml)	100 ml Tissue Lysis Reagent	289992
virotype TLR (250 ml)	250 ml Tissue Lysis Reagent	289993
virotype ASFV PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	281905

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

Product	Contents	Cat. no.
virotype BTV RT-PCR Kit (96)*	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280435
virotype BTV pan/8 RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	280445
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 Influenza A RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282605
virotype SBV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281605
bactotype Mycoplasma Mg/Ms PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	288105

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

Product	Contents	Cat. no.
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)	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
QIAcube (230 V)	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor	9001293
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 offer a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit

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

www.qiagen.com/Animal-and-Veterinary-Testing for more information about the *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

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Notes

The purchase of this product allows the purchaser to use it for amplification and detection of nucleic acid sequences for providing veterinary in vitro diagnostics. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

Limited License Agreement for *virotype* BVDV RT-PCR Kit

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

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.

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3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

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