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cador® BVDV RT-PCR Kit Handbook

Σ 48 (catalog no. 280303)

Σ 192 (catalog no. 280305)

For detection of BVDV in samples from cattle

REF 280303, 280305

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden

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Contents

Kit Contents

***cador* BVDV RT-PCR Kit**	**(48)**	**(192)**
Catalog no.	**280303**	**280305**
Number of preps	**48**	**192**
BVDV Master (blue cap)	2 x 370 µl	8 x 370 µl
BVDV 1 Control (red cap)	1 x 200 µl	1 x 200 µl
BVDV 2 Control (red cap)	1 x 200 µl	1 x 1000 µl
BVDV IC (green cap) IC	1 x 1000 µl	2 x 1000 µl
BVDV Mg-Sol (yellow cap) MG-SOL	1 x 400 µl	1 x 1000 µl
Water (PCR Grade - white cap)	1 x 1000 µl	1 x 1000 µl
Handbook HB	1	1

Intended Use

The *cador* BVDV RT-PCR Kit is intended for detection of BVDV in samples from cattle. The recommended starting material is viral RNA isolated from bovine plasma, serum, skin tissue (ear notches), milk pellets (cells), or EDTA blood (see "Viral RNA Isolation", page 9).

Symbols

Contains reagents for <N> tests

Legal manufacturer

Lot number

Use by date

Temperature limitations for storage

Handbook

REF	Catalog number
MAT	Material number
IC	Internal Control
MG-SOL	Magnesium solution
	For cattle samples

Storage

The components of the *cador* BVDV RT-PCR Kit should be stored at –15 to –30°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°C should not exceed a period of 5 hours.

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 *cador* BVDV RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *cador* BVDV RT-PCR Kit is a ready-to-use system for the detection of BVDV RNA using polymerase chain reaction (PCR).The BVDV Master contains reagents and enzymes for the reverse transcription and specific amplification of a highly conserved region of the BVDV genome. The amplicons are detected by measuring the FAM™ fluorescence (BVDV-1 and BVDV-2). In addition, the *cador* BVDV RT-PCR Kit contains a heterologous amplification system to identify possible PCR inhibition. This is determined by measuring the VIC® fluorescence due to amplification of an internal control (IC). The detection limit of the analytical BVDV RT PCR is not reduced. Two external positive controls (BVDV 1 Control and BVDV 2 Control) are supplied.

Principle

Pathogen detection by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is detected via 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 having to re-open the reaction tubes after the PCR run.

Pathogen Information

The Bovine Viral Diarrhea Virus (BVDV) is an animal pathogen with economic significance. It can be separated into two major genotypes, BVDV-1 and BVDV-2. Although genetically different, both BVDV 1 and BVDV 2 are pathogenic, and the major aspects of the pathogenesis of disease caused by the two genotypes are similar. BVDV infections can lead to diarrhea, hemorrhagic syndrome, or mucosal disease. Fertility dysfunction and abortion are also possible.

The virus is present in feces and mucous secretions and can be transmitted orally. The pathogens can be detected in blood, tissue (ear notches), and milk.

If an infection with a noncytopathic biotype occurs during a pregnancy, the fetus can develop a lifelong immunotolerance and become a chronic carrier of

the virus (persistently infected animals, PI animals). Identification of PI animals allows elimination of re-infection sources.

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.

- Nucleic acids isolation kit (see "Viral RNA Isolation", page 9)
- Pipets (adjustable)
- Sterile pipet tips with filters
- Vortexer
- Desktop centrifuge with rotor for 2 ml reaction 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
- 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
- Ice or cooling block

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 reagent past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

Viral RNA Isolation

We recommend using the QIAamp® Viral RNA Mini Kit (50) (QIAGEN, cat. no. 52904) for low-throughput, manual viral RNA purification from bovine plasma or serum.

For purification of viral RNA from skin tissue (ear notches), we recommend the RNeasy® Fibrous Tissue Mini Kit (50) (QIAGEN, cat. no. 74704). For purification of viral RNA from milk pellets (cells), a user-developed protocol is available using the RNeasy Mini Kit (cat. no. 74104 or 74106) or the RNeasy 96 Kit (cat. no. 74181 or 74182), with QIAshredder homogenizers (cat. no. 79654 or 79656).

When using isolation protocols with ethanol-containing wash buffers, such as the QIAamp Viral RNA Mini Kit or RNeasy Kits, it is important to carry out an additional centrifugation step before elution to remove any remaining ethanol. This prevents possible inhibition of PCR by carryover of ethanol. This extra step is included in the protocols in the relevant kit handbooks, marked as a recommended optional step.

Blood collection tubes coated with anticoagulants may inhibit downstream PCR. However, these inhibitors will be eliminated if the RNA isolation kits listed above are used. We recommend avoiding use of heparin blood.

The *cador* BVDV RT-PCR Kit is not compatible with phenol-based viral RNA isolation methods.

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

Carrier RNA

The use of carrier RNA is critical for the extraction efficiency and, consequently, for RNA yield from plasma and serum. Carrier RNA is included in the QIAamp Viral RNA Mini Kit. To increase the stability of the carrier RNA provided with the QIAamp Viral RNA Mini Kit, we recommend the following procedure, which differs from the procedure described in the kit handbook:

- Resuspend the lyophilized carrier RNA prior to first use of the extraction kit in 310 µl of the elution buffer provided with the kit (final concentration 1 µg/µl, do not use lysis buffer). Divide this carrier RNA solution into a number of suitably sized aliquots and store them at –15 to –30°C. Avoid repeated thawing (>2 x) of a carrier RNA aliquot.
- Before the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and internal control, where applicable, see "Internal Control", page 10) should be freshly prepared according to the following pipetting scheme.

Table 1. Preparation of lysis buffer and carrier RNA

Number of samples	1	12
Buffer AVL	560 µl	6720 µl
Carrier RNA (1 µg/µl)	5.6 µl	67.2 µl
Total volume	565.6 µl	6787.2 µl
Volume per extraction	560 µl	560 µl each

- Use the freshly prepared mixture of lysis buffer and carrier RNA immediately for extraction. Storage of the mixture is not possible.

Internal Control

An internal control (BVDV IC) is supplied. This allows the user to control the RNA isolation procedure and to check for possible PCR inhibition. For this application, add the internal control to the sample lysate (or lysis buffer) at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAamp Viral RNA Mini Kit the RNA is eluted in 60 µl AVE buffer. Therefore 6 µl of the internal control should be added to the sample lysate. The quantity of internal

control used depends only on the elution volume. The internal control and carrier RNA (see "Carrier RNA", page 10) should be added only to the mixture of lysis buffer and sample material or directly to the lysis buffer.

The internal control must not be added to the sample material directly. If added to the lysis buffer, please note that the mixture of internal control and lysis buffer–carrier RNA has to be freshly prepared and used immediately (storage of the mixture at room temperature or at 2–8°C for only a few hours may lead to internal control failure and a reduced extraction efficiency). Do not add the internal control and the carrier RNA directly to the sample material.

The internal control can optionally be used only to check for possible PCR inhibition. For this application, add 1 µl of the internal control and 1 µl BVDV Mg-Sol per reaction directly to 14 µl BVDV Master, as described in the protocol. For each PCR assay, use 15 µl of the master mix produced as described above* and add 10 µl of the purified sample. If you are preparing a PCR run for several samples, increase the volume of BVDV Master, BVDV Mg-Sol, and the internal control (BVDV IC) according to the number of samples (see "Protocol: PCR", page 12).

* The increase in volume caused by adding the internal control is not taken into account when preparing the PCR assay. The sensitivity of the detection system is not impaired.

Protocol: PCR

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 9–10.
- Take time to familiarize yourself with the chosen real-time PCR system before starting the protocol. See the user manual supplied with the instrument.
- Make sure that at least one positive control (BVDV 1 Control or BVDV 2 Control) as well as one negative control (Water, PCR grade) are included per PCR run.

Things to do before starting

- Before each use, all reagents need to be thawed completely at room temperature (15–25°C), mixed (by pipetting repeatedly up and down or by pulse vortexing), and centrifuged briefly. Then place all reagents in a cooling block at 2–8°C or on ice.

Procedure

1. If you want to use the internal control to monitor the RNA isolation procedure and to check for possible PCR inhibition, follow step 1a. If you want to use the internal control exclusively to check for PCR inhibition, follow step 1b.

1a. The internal control has already been added in the isolation procedure (see "Internal Control", page 10). In this case, prepare a master mix, in a cooling block at 2–8°C or on ice, according to Table 2.

The reaction mix typically contains all of the components needed for PCR except the sample. Prepare a volume of reaction mix at least 10% greater than that required for the total number of PCR assays to be performed.

Table 2. Preparation of master mix (internal control already added in the isolation procedure)

Number of samples	1	12	96
BVDV Master	14 µl	168 µl	1344 µl
BVDV Mg-Sol	1 µl	12 µl	96 µl
BVDV IC	0 µl	0 µl	0 µl
Total volume	15 µl	180 µl	1440 µl

1b. The internal control must be added directly to the BVDV Master. In this case, prepare a master mix, in a cooling block at 2–8°C or on ice, according to Table 3.

The reaction mix typically contains all of the components needed for PCR except the sample. Prepare a volume of reaction mix at least 10% greater than that required for the total number of PCR assays to be performed.

Table 3. Preparation of master mix (internal control not added in the isolation procedure)

Number of samples	1	12	96
BVDV Master	14 µl	168 µl	1344 µl
BVDV Mg-Sol	1 µl	12 µl	96 µl
BVDV IC	1 µl	12 µl	96 µl
Total volume	16 µl*	192 µl*	1536 µl*

* The increase in volume caused by adding the internal control is not taken into account when preparing the PCR assay. The sensitivity of the detection system is not impaired.

2. Pipet 15 µl of the master mix into each reaction tube or each well of a 96-well reaction plate. Then add 10 µl of the eluate from the RNA isolation (see Table 4). Correspondingly, 10 µl of at least one of the positive controls (BVDV 1 Control or BVDV 2 Control) must be used as a positive control and 10 µl of water (Water, PCR grade) as a negative control. Mix the solutions thoroughly by pipetting repeatedly up and down.

The external positive controls (BVDV 1 Control and BVDV 2 Control) are treated as previously purified samples and the same volume is used (10 µl). These positive controls provide a positive reference signal.

Table 4. Preparation of reaction mix

Component	Volume
Master mix	15 µl
Sample	10 µl
Total volume	25 µl

3. **Close the reaction tubes with the corresponding caps and centrifuge for 30 s at 1780 x *g* (4000 rpm) to collect the prepared reaction volume in the bottom of the tube.**
4. **Set the filters for the reporter and quencher dyes in the software of your thermal cycler according to Table 5. Select the green and yellow channels on the Rotor-Gene Q.**

Table 5. Filter settings for reporter and quencher

Pathogen/Internal Control	Reporter	Quencher
BVDV	FAM	TAMRA™
Internal control	VIC/HEX™/JOE™*	TAMRA

* Use the option appropriate for your thermal cycler.

5. **Run the real-time PCR protocol according to Table 6.**

Table 6. Real-time PCR protocol *

Temperature	Time	Number of cycles
50°C	30 min	1
95°C	10 min	1
95°C	30 s	45
55°C†	60 s	

† Fluorescence data collection.

* Suitable for the Rotor-Gene Q and ABI 7500 real-time cyclers. Other real-time PCR cyclers must be validated by the user.

Data Analysis and Interpretation

Interpretation of results

The following results (1a, 1b, or 1c) are possible.

1a. A fluorescent signal is identified in the FAM channel (green channel in the Rotor-Gene Q). The result of the analysis is positive: The sample contains BVDV RNA.

In this case, the identification of a fluorescent signal in the VIC (internal control; yellow channel in the Rotor-Gene Q) channel is not necessary since high initial concentrations of BVDV RNA (positive FAM/green fluorescence signal) can lead to a reduced or missing fluorescence signal of the internal control due to competition.

1b. No fluorescent signal is identified in the FAM channel (green channel in the Rotor-Gene Q). At the same time, a fluorescent signal from the internal control appears in the HEX channel (yellow channel in the Rotor-Gene Q). In the sample no BVDV RNA is identifiable. It can be considered negative.

In the case of a negative BVDV PCR result, the identified signal of the internal control rules out the possibility of PCR inhibition.

1c. No fluorescent signal is identified in the FAM channel (green channel in the Rotor-Gene Q) or in the VIC channel (yellow channel in the Rotor-Gene Q). No result can be concluded.

If no signal is detected in both the FAM/green (sample) and the VIC/yellow (internal control) channel, the result is inconclusive. The absence of a signal for the internal control indicates PCR inhibition and/or other malfunctions.

Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 17.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. 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).

	Comments and suggestions
No FAM fluorescence signal with positive controls (BVDV 1 Control and BVDV 2 Control)	
a) The selected detection dye for PCR data analysis does not comply with the protocol	For data analysis, select the detection dye FAM for the analytical BVDV RT-PCR and the detection dye VIC/HEX/JOE for the internal control RT-PCR.
b) Incorrect programming of the temperature profile	Compare the temperature profile with the protocol (see "Protocol: PCR", page 12).
c) Incorrect configuration of the PCR	Check your work steps (see "Protocol: PCR", page 12).
d) Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary. Make sure that kit components are stored as described in "Storage", page 5.
Weak or no signal of the internal control (HEX/JOE fluorescence signal) and simultaneous absence of a FAM fluorescence signal for the specific BVDV RT-PCR	
a) PCR conditions do not comply with the protocol	Check the PCR conditions (see "Protocol: PCR", page 12).

	Comments and suggestions
b) PCR inhibited	Make sure that you use a recommended isolation method (see "Viral RNA Isolation", page 9) and follow the manufacturer's instructions closely. Make sure that during the RNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see "Viral RNA Isolation", page 9).
c) RNA lost during extraction	If the internal control has been added to the extraction, the lack of a signal for the internal control can indicate loss of RNA during the extraction. Make sure that you use a recommended isolation method (see "Viral RNA Isolation", page 9) and follow the manufacturer's instructions closely.
d) Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary. Make sure that kit components are stored as described in "Storage", page 5.
A FAM fluorescence signal of the analytical RT-PCR with the negative controls	
a) Contamination during preparation of the PCR	Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after addition of the sample to be tested. Be sure to pipet the positive controls last. Ensure that work space and instruments are decontaminated at regular intervals.
b) Contamination during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that workspace and instruments are decontaminated at regular intervals.

Ordering Information

Product	Contents	Cat. no.
cador BVDV RT-PCR Kit (48)	For 48 reactions: BVDV Master, BVDV 1 Control, BVDV 2 Control, BVDV IC, BVDV Mg-Sol, Water (PCR Grade)	280303
cador BVDV RT-PCR Kit (192)	For 192 reactions: BVDV Master, BVDV 1 Control, BVDV 2 Control, BVDV IC, BVDV Mg-Sol, Water (PCR Grade)	280305
Related products		
QIAamp Viral RNA Mini Kit — for purification of viral RNA from cell-free body fluids		
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
QIAamp Viral RNA Mini Kit (250)	For 250 RNA preps: 250 QIAamp Mini Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-Free Buffers	52906
QIAcube — for fully automated sample preparation using QIAGEN spin-column kits		
QIAcube (110 V)† QIAcube (230 V)‡	Robotic workstation for automated purification of nucleic acids or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor§	9001292† 9001293‡

* Fully automatable on the QIAcube. See www.qiagen.com/MyQIAcube for protocols.

† US, Canada, and Japan.

‡ Rest of world.

§ Agreements for comprehensive service coverage are available; please inquire.

QIAGEN offer 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 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

Notes

www.qiagen.com

Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

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