



virotype[®] BVDV RT-PCR Kit Handbook

For detection of RNA from *Bovine Viral
Diarrhea Virus*

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-B 451

 24 reactions (cat. no. VT280373)

 96 reactions (cat. no. VT280375)

 480 reactions (cat. no. VT280377)



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Kit contents

virotype BVDV RT-PCR Kit	(24)	(96)	(480)
Cat. no.	VT280373	VT280375	VT280377
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 150 µl	2 x 150 µl
Negative Control (tube with blue cap)	1 x 25 µl	1 x 150 µl	1 x 300 µl
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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 licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 451) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols



Legal manufacturer



Lot number



Use by date



Temperature limitations for storage



Handbook



Catalog number



Material number



Protect from light



For cattle samples

Quality control

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

Storage

The components of the virotype BVDV 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 from your local sales representative or by Email request under **compliance@indical.com**.

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

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.

The kit uses two specific primer/probe combinations: one for pestiviruses yielding FAM™ fluorescence and one for the internal control yielding HEX™ fluorescence. This internal control excludes the possibility of false negative results.

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. INDICAL offers a range of validated kits for the extraction of RNA and DNA from animal samples.

- QIAamp® cador® Pathogen Mini Kit
- cador® Pathogen 96 QIAcube® HT Kit
- MagAttract® 96 cador® Pathogen Kit

Furthermore, the following extraction kits can be purchased from QIAGEN GmbH

- 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 the use of virotype Tissue Lysis Reagent is recommended. 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
- 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

- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Real-time cycler with appropriate fluorescent channels
- Appropriate software for chosen real-time cycler
- Appropriate strip tubes and caps or 96-well optical microplate with optical sealing film or cover for chosen 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 as 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 detection of RNA from *Bovine Viral Diarrhea Virus*

Important points before starting

- Please read „Important notes“ on page 9 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	Number of reactions			
	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 dyes in the software of your thermal cycler according to Table 3.

Table 3. Filter settings for the reporter

Pathogen/ internal control	Reporter	Rotor-Gene Q
BVDV	FAM	green
Internal Control	HEX/ JOE ¹	yellow
Passive reference ²	ROX	-

¹ Use the option appropriate for your thermal cycler.

² Internal reference for use on Applied Biosystems® 7500

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

Table 4. Real-time RT-PCR protocol for BVDV

Step	Temperature	Time	Number of cycles
Reverse Transcription	50°C	20 min	1
Initial Activation	95°C	15 min	1
3-step cycling			
Denaturation	95°C	30 s	
Annealing*	57°C	45 s	40
Extension	68°C	45 s	

* Fluorescence data collection. Approximate run time 2h 38min (Rotor-Gene Q)

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 $C_T^1 < 36$. 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 17.

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 the HEX channel.
- The Positive Control yields a signal in both the FAM and the HEX channel.
- 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).

¹ Threshold cycle (CT) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence

The 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 the HEX channel.
- 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 criteria are met:

- 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	FAM (BVDV)	HEX (IC)
BVDV positive	X	X
BVDV positive (strong positive)	X	
BVDV negative		X
inconclusive		

* 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 15.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.

Visit **www.indical.com** for more information about bactotype, cadov, cattletype, flocktype, pigtype and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

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Change index

Handbook	Version	Change
HB-1577-EN-006	October 2018	amendment in „Interpretation of Results“
HB-1577-EN-005	May 2018	INDICAL design

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