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virotype® BTV pan/8 2.0 RT-PCR Kit

Handbook

For detection of RNA from *Bluetongue Virus* (BTV) and European serotype BTV-8

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 114

REF 100 reactions (Cat. no. VT280465)

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

INDICAL BIOSCIENCE

Contents

Kit contents

virotype BTV pan/8 2.0 RT-PCR Kit **Cat. no.** **Number of reactions**	**(100)** **VT280465** **100**
Master Mix (tube with orange cap) includes enzymes, primers, and probes	1 x 800 µl
Positive Control (tube with red cap)	1 x 150 µl
Negative Control (tube with blue cap)	1 x 150 µl
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Intended use

The virotype BTV pan/8 2.0 RT-PCR Kit is intended for the detection of *Bluetongue Virus* (BTV) RNA and differentiation of European serotype BTV-8 specifically, in ruminant whole blood and blood pools (preferred with anticoagulants, for example EDTA-blood) 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 114) 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 samples from cattle, sheep and goats

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of virotype BTV pan/8 2.0 RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the virotype BTV pan/8 2.0 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 (> 3x), 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

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 36 known serotypes including atypical BTV. 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 becomes cyanotic (Bluetongue).

BTV serotype 8 (BTV-8) is of epidemiological importance in Central Europe and cause of recent major 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 PCR, the amplified product is identified 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/8 2.0 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/8 2.0 RT-PCR Kit uses three specific primer/probe combinations:

- FAM™ fluorescence for RNA of all known BTV serotypes (pan BTV)
- Cy®5 fluorescence for RNA of European serotype 8 (BTV-8)
- JOE™ fluorescence for the endogenous Internal Control (EC; β-actin present within the sample)

A Positive Control contains BTV-8 RNA and 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/8 2.0 RT-PCR Kit can be used for the detection of BTV RNA from ruminant whole blood (preferred with anticoagulants, e.g., EDTA-blood) and tissue samples (spleen, lymph nodes) from cattle, sheep and goats.

Due to the high sensitivity of the test, pools of up to 10 individual blood samples may be analyzed. However, the optimum pool size depends on the regional prevalence for BTV.

Note: For use in Germany the specifications described in the „*Amtliche Methodensammlung*“ apply.

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 from animal samples.

Extraction based on magnetic beads:

- **IndiMag® Pathogen Kit** (SP947457)
- **IndiMag Pathogen Kitw/o plastics** (SP947257)
- **IndiMag Pathogen IM48 Cartridge** (SP947654P608, SP947654P224)
- **IndiMag Pathogen KF96 Cartridge** (SP947855P196)

Extraction based on spin columns:

- **IndiSpin® Pathogen Kit** (SP54104, SP54106)
- **IndiSpin QIAcube® HT Pathogen Kit** (SP54161)

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 further information on automated and manual extraction of BTV RNA from different sample types, refer to the respective handbook or contact INDICAL Support at **support@indical.com**.

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, containing all the components of the reaction except for the pathogen template. 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, e.g., the correct setup of the reaction mix. Use 5 µl of the Positive Control provided with

the virotype BTV pan/8 2.0 RT-PCR Kit to test for successful amplification of the target.

Extraction and amplification control

For increased process safety and convenience, one extraction and amplification control assay is included in the test kit.

An endogenous internal control (EC) detects the β-actin gene present within the sample. This allows extraction and amplification to be monitored.

Protocol: Real-time RT-PCR for detection of RNA from *Bluetongue Virus* and BTV-8

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.
- Before use, spin the reagents briefly.
- Maintain reagents on ice or in a cooling block during PCR setup.

Procedure

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

   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 and Controls 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. Before use, mix the Master Mix by inverting 5 times or until mixed thoroughly, then centrifuge briefly to collect the fluids.
5. Pipet 8 µl of the Master Mix into each reaction tube. Thus, the final volume of a test is 13 µl (Table 1).

Table 1. Preparation of reaction mix

Component	**Volume**
Master Mix	8 µl
Sample	5 µl
Total volume	**13 µl**

6. Close the reaction tubes with the corresponding caps.
7. Invert the closed tubes several times until mixed thoroughly and spin down briefly.
8. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for the reporter

Pathogen/ internal control	Reporter
pan BTV	FAM
BTV-8	Cy5
Endogenous internal control (EC)	HEX/ JOE™[1]
Passive reference[2]	ROX™

1 Use the option appropriate for your thermal cycler.
2 Internal reference for use with ABI PRISM® Sequence Detection Systems (Applied Biosystems®)

9. Run the real-time PCR protocol according to Table 3.

Table 3. Real-time RT-PCR protocol for BTV pan/8 2.0

Step	Temperature	Time	Number of cycles
Reverse Transcription	50°C	10 min	1
Initial Activation	95°C	2 min	1
2-step cycling			
Denaturation	95°C	5 s	40
Annealing/ Extension*	60°C	30 s	

* Fluorescence data collection, approximate run time 67 min (CFX96, Bio-Rad™)

Data analysis and interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in the FAM and HEX/ JOE channels with a C_T[1] < 35. If no FAM and no Cy5 signals of the Positive Controls are measured the denaturation 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 4 on page 17.

The sample is positive for pan BTV and BTV-8, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the FAM, Cy5 and HEX/JOE channel.
- The Positive Control yields a signal in all channels.
- The Negative Control yields no signal in any of the channels.

Note that very high concentrations of BTV-8 RNA or presence of inhibitors in the sample may lead to a reduced HEX/JOE signal or no HEX/JOE signal due to competition with the internal control.

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

The sample is positive for pan BTV and negative for BTV-8, and the assay is valid, if the following criteria are met:

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

Note that very high concentrations of BTV-8 RNA or presence of inhibitors in the sample may lead to a reduced HEX/JOE signal or no HEX/JOE signal due to competition with the internal control.

The sample is negative for both, pan BTV and BTV-8, and the assay is valid, if the following criteria are met:

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

A positive HEX/JOE 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 criteria are met:

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

If no signal is detected in the FAM (pan BTV), Cy5 (BTV-8) and the HEX/JOE (endogenous Internal Control, EC) channels, the result is inconclusive. The absence of a signal for the housekeeping gene indicates strong PCR inhibition and/or other malfunctions, e.g., during extraction.

To check for inhibition, we recommend 1:5 dilution of the sample RNA in nuclease-free water, to repeat the RNA extraction procedure, or 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.

Table 4. Results interpretation table*

Sample result	FAM (pan BTV)	Cy5 (BTV-8)	HEX (EC)
pan BTV positive	X		(X)
pan BTV and BTV-8 positive	X	X	(X)
BTV 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 the FAM, Cy5 and HEX/JOE channels. The Negative Control must yield no signal in any 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 afosa, bactotype, cador, cattletype, flocktype, pigtype, Svanovir and virotype products.

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

Notes

Notes

Limited License Agreement for virotype BTV pan/8 2.0 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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Handbook	Version	Change
HB-2578-EN-001	July 2022	Product launch

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