VetMAX™ BTV4 IAH Typing Kit

TaqMan™ real-time RT-PCR for detection of BTV4 (Bluetongue Virus type 4)

Catalog Number BTV4GIAH50

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IMPORTANT! Product registered with the French National Reference Laboratory – Anses Maisons-Alfort. Specific requirements apply for the use of the kit in diagnostic procedures in France, see Appendix A.

Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time RT-PCR (RNA) - Duplex - IPC Endogenous	Bovine Small ruminants (sheep, goats)	Blood (in EDTA tubes) Spleen or aborted fetus (spleen, liver, heart)	Individual

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

⚠ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Information about the product

Description of the product

Bluetongue disease is a non-contagious, insect-borne infectious disease in sheep, included on the A list of the International Office of Epizootics. It is caused by infection with Bluetongue virus (BTV), a virus in the Reoviridae family, Orbivirus genus. To date, 35 different BTV serotypes have been identified.

BTV is basically considered dangerous for sheep: it leads to major morbidity and high mortality. The virus also infects cattle, goats and other wild ruminants, but only rarely leads to clinical manifestations in these species (Lefèvre and Desoutter, 1998).

The virus is nearly always transmitted by the infectious bite of a small, blood-sucking fly belonging to the Ceratopogonidae family, *Culicoides* genus. There are more than 1,400 species of *Culicoides*, but not all are capable of transmitting the virus. *Culicoides* becomes infected after feeding on the blood of an infected animal, then the virus reproduces until a sufficient titer is reached, enabling transmission to other susceptible animals.

The **Applied Biosystems™ VetMAX™ BTV4 IAH Typing Kit** is a BTV type 4 molecular diagnostic tool. This is a second-line kit that, after BTV analysis of the positive group, enables specific detection of the BTV4 virus through RT-PCR technology in real time.

Each RNA sample is analyzed in a single well: the same well is used to specifically detect the viral RNA of BTV4 and an IPC (Internal Positive Control). A positive IPC signifies both successful extraction and the absence of PCR inhibitors in the sample.

This kit can be used on viral RNA extracted from whole blood (in EDTA tubes), spleen, or organs of aborted animals.

Complete protocols for viral RNA extraction from these matrices are available upon request from Technical Support.

Kit contents and storage

The **VetMAX™ BTV4 IAH Typing Kit** contains components that can be used for detecting both BTV4 and an IPC. Upon receipt, the kit should be stored unopened at **–30°C to –10°C.** After the initial use, follow the recommendations for storage of each component in the following table:

Component	Description	Volume (50 tests)	Storage	
			Upon receipt	After initial use
3 - Mix BTVEUG4 (Blue tube)	Mix for TaqMan™ RT-PCR. Contains: • The detection system for the BTV4 target, including a TaqMan™ probe labeled **FAM™ – NFQ** (Non-Fluorescent Quencher). • The detection system for the IPC, including a TaqMan™ probe labeled **VIC™ – NFQ** (Non-Fluorescent Quencher). • Buffer, reverse transcriptase, and real-time PCR enzyme.	1,000 µL	–30°C to –10°C	–30°C to –10°C
4a - EPC BTVEUG4 (Blue tube)	**External Positive Control:** BTV4 positive control. It uses nucleic acid **already extracted** for denaturation, then amplification, during the real-time RT-PCR.	90 µL	–30°C to –10°C	–30°C to –10°C

Extraction and amplification controls

The **VetMAX™ BTV4 IAH Typing Kit** contains one control used to validate the amplification of the viral RNAs:

4a - EPC BTVEUG4: positive control of BTV4

A positive control, **already extracted,** for amplification during real-time RT-PCR.

A positive result within the specified C_t range enables amplification validation of the BTV4 target by real-time RT-PCR.

In France, follow the additional requirements described in Appendix A.

Validation of nucleic acid extraction for each sample is done by detection of an **endogenous IPC** (Internal Positive Control), **present in each sample**.

A positive IPC result for a sample validates the extraction of that sample, whether the sample is positive or negative for the pathogen being investigated, eliminating false negatives due to PCR inhibition.

We recommend including two negative controls to confirm correct analysis:

NCS: negative extraction control

This control consists of DNase/RNase-free water or a sample known to be free of target pathogen that undergoes the same treatment (nucleic acid extraction and real-time RT-PCR) as the samples.

A negative result of BTV4 and endogenous IPC confirms the absence of contamination during the extraction and the real-time RT-PCR.

NC: negative amplification control

This control consists of 20 µL of real-time RT-PCR mix and 5 µL DNase/RNase-free water that undergoes real-time RT-PCR.

A negative result of BTV4 and IPC confirms the absence of contamination during real-time RT-PCR reaction preparation.

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

- Adjustable micropipettes (range of 1 µL to 1,000 µL) with DNase/RNase-free filtered tips
- DNase/RNase-free water
- 1X TE buffer
- 1X PBS buffer
- **Heating block** capable of reaching **95°C**
- A real-time **PCR thermal cycler** capable of detecting the following fluorophores:
  - **FAM**™ (maximum emission: λ515 nm)
  - **VIC**™ (maximum emission: λ554 nm)
- Optical-quality consumables compatible with the thermal cycler used: PCR 96-well plates, PCR strips (8 or 12 wells), microtubes or capillaries; suitable plate covers or caps for capping

Analysis procedure

The reaction volume of the real-time RT-PCR is 25 µL:

- **3 - Mix BTVEUG4:** 20 µL per reaction
- **Extracted RNA:** 5 µL per reaction

Extraction of viral RNA

RNA must be extracted from the samples prior to real-time RT-PCR analysis.

NOTE: To learn about compatible and validated extraction methods for the VetMAX™ BTV4 IAH Typing Kit, please contact Technical Support.

Denaturation of RNA

1. Add the RNA to be denatured into the wells of a PCR plate or strip. Include 10% overage of the extracted RNA for all reactions to ensure that sufficient RNA is present after denaturation.
2. Cap the wells containing the RNA.
3. Heat for 3 minutes to between +92°C and +98°C in a thermal cycler or heating block.
4. Store the denatured RNA at between +2°C and +8°C on crushed ice or on a refrigerated block until use.

Preparation of the real-time RT-PCR

1. Create an analysis plan for distribution of the mixes and samples. Keep the positive control (EPC) away from the other samples if possible.
2. Thaw **3 - Mix BTVEUG4** between **+2°C and +8°C, on ice** or on a refrigerated rack.
3. Homogenize the **3 - Mix BTVEUG4** tube by gentle agitation, then centrifuge briefly.
4. Add **20 µL of 3 - Mix BTVEUG4** to each well on the PCR plate, PCR strip or capillary used.
5. Add RNA from samples and controls to the real-time RT-PCR mix solution according to the following preset analysis plan:

Type of analysis	Component	Sample volume
Sample for analysis	RNA extracted from the sample and denatured	5 µL
Positive amplification control	Denatured **4a - EPC BTVEUG4**	5 µL
Negative extraction control (NCS)	Extracted and denatured NCS	5 µL
Negative amplification control (NC)	DNase/RNase-free water	5 µL

6. Cover the PCR plate, PCR strips or capillaries with an adhesive plate cover or suitable caps.

In France, follow the additional requirements described in Appendix A.

Real-time RT-PCR amplification

1. Set up the following detectors on the thermal cycler:

	Reporter	Quencher
BTV4	FAM™	NFQ (Non-Fluorescent Quencher)
IPC BTV4	VIC™	NFQ (Non-Fluorescent Quencher)
Passive reference: ROX™[1]		

[1] The ROX™ fluorophore must be entered for real-time RT-PCR analysis if the thermal cycler is capable of detecting them. For all other thermal cyclers, absence of the ability to detect these fluorophores does not compromise the analysis by real-time RT-PCR.

2. Set up the **BTV4** and **IPC BTV4** detectors for each well used in the analysis.
3. Set up one of the following real-time RT-PCR programs for the analysis:

Table 1 Standard method (for use with samples purified using a standard script on a KingFisher™ instrument)

	Step repetitions	Temperature	Duration
Step 1	×1	45°C	10 minutes
Step 2	×1	95°C	10 minutes
Step 3	×40	95°C	15 seconds
		60°C[1]	45 seconds

[1] Collection of fluorescence data during the 60°C – 45 seconds stage.

Table 2 Express method (for use with blood samples purified using an express script on a KingFisher™ instrument)

	Step repetitions	Temperature	Duration
Step 1	×1	48°C	5 minutes
Step 2	×1	97°C	5 minutes
Step 3	×40	97°C	2 seconds
		60°C[1]	35 seconds

[1] Collection of fluorescence data during the 60°C – 35 seconds stage.

4. Place the PCR plate, PCR strips or capillaries in the thermal cycler and start the real-time RT-PCR.

Interpretation of results

Raw data analysis

Please refer to the thermal cycler manufacturer's recommendations for raw data analysis.

1. Set the threshold lines separately for each target.
2. Interpret the results based on the C_t values of the samples for each detector according to the following recommendations.

Validation

The test is validated if the following criteria are met:

	BTV4 detector	IPC BTV4 detector	Validation
EPC BTVEUG4	$C_t = C_{t\,QC}$ BTV4 of **4a- EPC BTVEUG4** ± 3Ct[1]	$C_t < 40$ or $C_t > 40$[2]	RT-PCR validated
NCS	$C_t > 40$	$C_t > 40$	Extraction validated
NC	$C_t > 40$	$C_t > 40$	RT-PCR reagents validated

[1] Please refer to the values shown for "EPC", in the Certificate of Analysis of the group used for the test.
[2] The IPC value in the EPC should not be used for test validation.

Interpretation of results

NOTE: Specific requirements apply for the use of the kit in diagnostic procedures in France, see Appendix A.

For each sample analyzed, the results should be interpreted as shown below:

BTV4 detector	IPC BTV4 detector	Interpretation
$C_t < 40$	$C_t < 40$ or $C_t > 40$	BTV4 detected
$C_t > 40$	$C_t < 40$	BTV4 not detected
$C_t > 40$	$C_t > 40$	Not validated[1]

[1] The sample will be returned as not validated due to the negative IPC.

In France, follow the additional requirements described in Appendix A.

Retest samples with invalid results

If an invalid result is obtained for a sample, we recommend performing one of the following procedures according to the quality of the RNA eluate.

For...	Do this...
Easy-to-pipet (low viscosity) eluates	1. Dilute the RNA eluate 1:5 in 1X TE buffer. 2. Denature the diluted RNA for 3 minutes at 92°C to 98°C. 3. Repeat the real-time RT-PCR procedure with 5 µL of the denatured RNA, then interpret the results as indicated. • If the real-time RT-PCR result is positive or negative for BTV, and the IPC result is acceptable (C_t IPC < 35), the result is validated. • If the real-time RT-PCR result remains invalid (C_t IPC ≥ 35), dilute the sample 1:2 in 1X PBS buffer, then repeat the nucleic acid extraction procedure.
Difficult-to-pipet (high viscosity) eluates	1. Dilute the sample 1:2 in 1X PBS buffer. 2. Repeat the nucleic acid extraction procedure. 3. Denature the RNA eluate for 3 minutes at 92°C to 98°C. 4. Repeat the real-time RT-PCR procedure with 5 µL of the denatured RNA, then interpret the results as indicated. • If the real-time RT-PCR result is positive or negative for BTV, and the IPC result is acceptable (C_t IPC < 35), the result is validated. • If the real-time RT-PCR result remains invalid (C_t IPC ≥ 35), repeat the analysis on a new sample.

Appendix A – Additional requirements applicable in France

Product registered with the French National Reference Laboratory - Anses Maisons-Alfort, for its use in France. The following specific requirements apply:

Extraction and amplification controls

As part of a COFRAC accreditation in accordance with the NF U47-600-1 standard, it is recommended to use an internal reference material, also referred to as a "sentinel", in each extraction series for validation of extraction, purification, and amplification steps. This sentinel must be prepared as described in Appendix B of the NF U47-600-1 standard and is to be processed as a sample or as an EPC.

Interpretation of results

The French National Reference Laboratory requires retesting of any samples showing an IPC with a C_t ≥ 35 (see "Retest samples with invalid results").

Documentation and support

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Revision history of Pub. No. MAN0008695 (English)

Revision	Date	Description
C.0	23 February 2021	•Updated to include a new express thermal protocol. •Updated "Retest samples with invalid results". •Updated "Description of the product". •Added Appendix A, "Additional requirements applicable in France". •Updated the limited license information.
B.0	19 February 2018	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	17 January 2014	Baseline for revision history

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