VetMAX™ IBR gB Kit

Real-time PCR TaqMan® for detection of BHV1 (Bovine Herpes Virus type 1)

Catalog Number IBRP50

Doc. Part No. 100020375 **Pub. No.** MAN0008780 **Rev.** D.0

Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time PCR (DNA) – Duplex – Endogenous IPC	Bovine Sheep Goat	Nasal swabs Pulmonary lavages Semen	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

The **Applied Biosystems™ VetMAX™ IBR gB Kit** is a molecular diagnostic tool that enables detection by real-time PCR of BHV1 virus (Bovine Herpes Virus type 1) by targeting the gene encoding for gB protein. It can be used in cases of symptomatic infections characteristic of IBR (Infectious Bovine Rhinotracheitis), but it does not enable detection of latent-phase BHV1.

Each DNA sample obtained after extraction is analyzed in a single-well plate; the same plate is used to specifically detect the viral DNA of BHV1 and to detect an IPC (Internal Positive Control). A positive IPC reflects both the efficiency of extraction and the absence of inhibitor in the samples.

It can be used with viral DNA extracted from **nasal swabs**, **pulmonary lavages**, and **semen**.

Complete protocols for viral DNA extraction from these matrices are available on request from Technical Support.

Kit contents and storage

The **VetMAX™ IBR gB Kit** contains reagents for detection in duplex of BHV1 and an internal positive control (IPC). Upon receipt, the whole kit must be stored between **–30°C and –10°C**. After initial use of a component, store it according to the following recommendations:

Component	Description	Volume (50 reactions)	Storage	
			Upon receipt	After initial use
3 - Mix IBR (Green tube)	Mix for TaqMan® PCR. Contains: • The detection system for the BHV1 target, including a TaqMan® probe labeled with **FAM™ – TAMRA™**. • The detection system for IPC, including a TaqMan® probe labeled **VIC™ – NFQ** (Non-Fluorescent Quencher). • The buffer and the real-time PCR enzyme.	2 × 500 µL	–30°C to –10°C	–30°C to –10°C
4a - EPC IBR (Brown tube)	**External Positive Control:** BHV1 positive control. It consists of **already extracted** nucleic acid to be amplified during real-time PCR.	90 µL	–30°C to –10°C	–30°C to –10°C

Extraction and amplification controls

The **VetMAX™ IBR gB Kit** contains 1 control, enabling validation of the extraction and the amplification of the viral DNA.

4a - EPC IBR: positive control of BHV1

Already extracted positive control to be amplified during real-time PCR.

A positive result within the specified C_t range enables amplification validation of the BHV1 target by real-time PCR.

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 with a compliant value in a sample validates the extraction of this sample, whether positive or negative for the target pathogen: elimination of false negatives and verification of the inhibitor effect.

We recommend including two negative controls to confirm correct analysis:

NCS: negative extraction control

This control consists of components used in the extraction without addition of the sample (the sample volume can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water), which undergoes the same treatment (nucleic acid extraction then real-time PCR) as the samples.

A negative result of BHV1 and endogenous IPC confirms the absence of contamination during the extraction and the real-time PCR.

NC: negative amplification control

This is the amplification mix deposited on the plate during the preparation of the real-time PCR, with 5 µL of DNase/RNase-free water added to adjust the reaction to 25 µL.

A negative result of BHV1 and IPC confirms the absence of contamination during real-time PCR reaction preparation.

Materials required but not provided

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

- Precision micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filtered tips
- DNase/RNase-free water
- 1X TE buffer
- 1X PBS buffer
- A real-time PCR thermal cycler capable of detecting the following fluorophores:
  - FAM™ (emission maximum: λ515 nm)
  - VIC™ (emission maximum: 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 real-time PCR reaction volume is 25 µL:

- **3 - Mix IBR:** 20 µL per analysis
- **Extracted DNA:** 5 µL per analysis

Extraction of bacterial DNA

It is necessary to isolate the DNA from the samples for real-time PCR analysis.

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

Preparation of the real-time 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 IBR** between **2°C and 8°C on ice** or on a refrigerated rack.
3. Mix **3 - Mix IBR** by gently shaking the tube, then centrifuge briefly.
4. Add **20 µL of 3 - Mix IBR** to each well on the PCR plate, PCR strip, or capillary used.
5. Add DNA from samples and controls to the reaction mix, according to the following preset analysis plan:

Type of analysis	Component	Sample volume
Sample for analysis	DNA extracted from the sample	5 µL
Positive amplification control	**4a - EPC IBR**	5 µL
Negative extraction control (NCS)	Extracted 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.

Amplification by real-time PCR

1. Create the following detectors on the thermal cycler:

	Reporter	Quencher
IBR (BHV1)	FAM™	TAMRA™[1]
IPC IBR	VIC™	NFQ
Passive reference: ROX™[1]		

[1] The TAMRA™ and ROX™ fluorophores are required for real-time PCR analysis if the thermal cycler is capable of detecting them. For other thermal cyclers, absence of the ability to detect these fluorophores does not affect the analysis by real-time PCR.

2. Assign the **IBR** detector and the **IPC IBR** detector to each sample well used in the analysis.
3. Create the following real-time PCR program for the analysis:

	Step repetitions	Temperature	Duration
Step 1	×1	50°C	2 minutes
Step 2	×1	95°C	10 minutes
Step 3	×40	95°C	15 seconds
		60°C[1]	1 minute

[1] Collection of fluorescence data during the 60°C – 1 minute stage

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

Analysis of the results

Analysis of the raw data

Refer to the recommendations of the thermal cycler manufacturer for the analysis of the raw data.

1. Position the threshold limits separately for each target of the real-time PCR.
2. For each detector, interpret the results according to the sample C_t values obtained as recommended below.

Validation

The run is validated if the following criteria are met:

	IBR detector	IPC IBR detector	Validation
EPC IBR	$C_t = C_{t\ qc}$ IBR of **4a - EPC IBR** $\pm 3C_t$[1]	$C_t < 40$ or $C_t > 40$[2]	PCR validated
NCS	$C_t > 40$	$C_t > 40$	Extraction validated
NC	$C_t > 40$	$C_t > 40$	PCR components validated

[1] Please refer to the values shown in section 2.1 "EPC" of 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

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

IBR detector	IPC IBR detector	Interpretation
$C_t < 40$	$C_t < 40$ or $C_t > 40$	BHV1 detected
$C_t > 40$	$C_t < 40$	BHV1 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.

Procedure for handling non-validated samples

1. Dilute the sample DNA at a 1:10 dilution in 1X TE buffer.
2. Perform a new PCR analysis on 5 µL of this dilution.
3. If the diluted DNA is positive for BHV1 or negative for BHV1 with a compliant IPC result, the obtained result is then validated.
4. If the diluted DNA is negative for BHV1 with a non-compliant IPC result, the obtained result is still not validated. In this case, repeat the nucleic acid extraction using the sample pre-diluted 1:10 in 1X PBS buffer before extraction.
5. If the result is still not validated, repeat the analysis on a new sample.

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.

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Revision history of Pub. No. MAN0008780 (English)

Revision	Date	Description
D.0	22 December 2016	Corrected all C_t cutoff values to 40. Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
C.0	March 2015	Correction of fluorophores and storage conditions
B.0	October 2014	Correction of the type of IPC
A.0	May 2014	New document

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