VetMAX™ Schmallenberg Virus Kit

TaqMan™ real-time RT-PCR for the detection of SBV (Schmallenberg Virus)

Catalog Number SBVS50

Doc. Part No. 100020435 **Pub. No.** MAN0008328 **Rev.** C.0

Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time RT-PCR (RNA) – Duplex – Endogenous IPC	Bovine Small ruminants (sheep, goats)	Brain tissue Blood (EDTA tubes) Serum	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™ Schmallenberg Virus Kit** is a molecular diagnostic tool for SBV. It specifically detects the S gene of SBV using real-time reverse transcription PCR.

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

The kit can be used on viral RNA extracted from **brain tissue, whole blood** collected in EDTA tubes or **serum**. Viral detection is preferably performed on brain from aborted animals, but the virus can also be detected in blood (Source: FLI – German National Reference Laboratory). In the absence of further information on the period of viremia, which seems very short (2 to 3 days), and on the location of the virus in the living animal, we recommend working preferentially with viral RNA extracted from brain tissue.

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

Kit contents and storage

The **VetMAX™ Schmallenberg Virus Kit** contains reagents for the detection in duplex of SBV and an IPC. Upon receipt, the whole kit must be stored at **–30°C to –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 SBVS (Green tube)	Mix for TaqMan™ RT-PCR. Contains: • The detection system for SBV target: forward and reverse primers, as well as a TaqMan™ probe labeled with **FAM™ - NFQ** (NFQ = Non-Fluorescent Quencher). • The detection system for IPC: forward and reverse primers, as well as a TaqMan™ probe labeled with **VIC™ - TAMRA™**. • Buffer, reverse transcriptase and PCR enzyme.	2 × 500 µL	–30°C to –10°C	–30°C to –10°C
4a - EPC SBVS (Brown tube)	**External Positive Control:** Positive control for SBV. 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™ Schmallenberg Virus Kit** contains one control used to validate the amplification of viral RNA:

4a - EPC SBVS: positive control for SBV

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

A positive result within the specified C_t range enables amplification validation of the SBV target by real-time RT-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 for a sample validates the extraction of that sample, whether the sample is positive or negative for the pathogen being investigated, enabling elimination of false negatives and verification of inhibition, if present.

We recommend including two negative controls to confirm correct analysis:

NCS: negative extraction control

This control is composed of reagents used in the extraction without addition of the sample (the volume of the sample can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water) that undergoes the same treatment as the samples: nucleic acid extraction (with addition of the IPC) and real-time RT-PCR.

A negative result for SBV and IPC enables the validation of the absence of contamination during the extraction and the real-time RT-PCR.

NC: negative amplification control

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

A negative result for SBV and IPC enables validation of 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**.

- 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™ (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 real-time RT-PCR reaction volume is 25 µL:

- **3 - Mix SBVS**: 20 µL per analysis
- **Extracted RNA:** 5 µL per analysis

Extraction of viral RNA

RNA must be isolated from the samples for real-time RT-PCR analysis.

NOTE: For information about extraction methods that are compatible with and validated for the VetMAX™ Schmallenberg Virus Kit, please contact Technical Support.

Preparation of the real-time RT-PCR reactions

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 SBVS** at **2°C to 8°C on ice** or in a refrigerated rack.
3. Mix **3 - Mix SBVS** by gentle agitation, then briefly centrifuge.
4. Add **20 µL of 3 - Mix SBVS** to each PCR plate well, PCR strip or capillary.
5. Add the RNA from samples and controls to the reaction mix, according to the following preset analysis plan:

Type of analysis	Component	Sample volume
Sample for analysis	RNA extracted from the sample	5 µL
Positive amplification control	**4a - EPC SBVS**	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 RT-PCR

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

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

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

2. Set up the **SBV** and **SBV IPC** 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 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, the PCR strips or the capillaries in the thermal cycler and run the real-time RT-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 test is validated if the following criteria are met:

	SBV detector	IPC SBV detector	Validation
EPC SBVS	$C_t = C_{t\,QC}$ SBV of **4a - EPC SBVS** ± 3C_t[1]	$C_t < 40$ or $C_t > 40$[2]	PCR validated
NCS	$C_t > 40$	$C_t < 40$	Extraction validated
NC	$C_t > 40$	$C_t > 40$	Validated PCR components

[1] Refer to the values shown for "EPC" in the Certificate of Analysis of the lot 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:

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

[1] The sample will be considered as not validated due to the non-compliant IPC value.

Procedure for handling non-validated samples

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

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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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Translated from the French Pub. No. MAN0007811 Rev. C.0.

The information in this guide is subject to change without notice.

Revision history of Pub. No. MAN0007811 (French)

Revision	Date	Description
C.0	18 December 2020	Updated to include a new express thermal protocol and to change the number of cycles for the standard protocol.
B.0	9 March 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	November 2015	Modification of sample matrices
2.0	June 2013	Correction of trademarks
1.0	April 2013	New document