February 2015

virotype® SBV RT-PCR Reagent Handbook


96 (catalog no. 281615)
480 (catalog no. 281617)*

For identification of Schmallenberg virus RNA by real-time RT-PCR

REF 281615, 281617*
For research use only.
USA/Canada: not for use in diagnosis of disease in animals.


QIAGEN Leipzig GmbH, Deutscher Platz 5b, 04103 Leipzig, Germany

* Available only on request.

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

In addition, QIAGEN provide high-quality, easy-to-use, and sensitive molecular solutions to enable animal pathogen identification and animal pathogen research. The QIAGEN animal pathogen portfolio includes a broad range of pathogen-specific PCR reagents. For more information, visit www.qiagen.com/Animal-Pathogens-and-Genotyping.

Contents

Reagent Contents

***virotype* SBV RT-PCR Reagent**	**(96)**	**(480)**
Catalog no.	**281615**	**281617***
Number of reactions	**96**	**480**
Master Mix (tube with orange cap) includes enzymes, primers, and probes	2 x 980 µl	6 x 1625 µl
Positive Control (tube with red cap)	1 x 70 µl	2 x 50 µl
Handbook	1	1

* Available only on request.

Intended Use

The *virotype* SBV RT-PCR Reagent is intended for identification of RNA from Schmallenberg virus (SBV). The *virotype* SBV RT-PCR Reagent is for research use only. USA/Canada: not for use in diagnosis of disease in animals.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant RNA experiments, or to other applicable guidelines.

Symbols

	Contains reagents for <N> tests
	Legal manufacturer
	Lot number
	Use by date
	Temperature limitations for storage
	Catalog number
	Material number
	Protect from light

Storage

The components of the *virotype* SBV RT-PCR Reagent should be stored at –15 to –30°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 online in convenient and compact

PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

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

Quality Control

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

Introduction

The *virotype* SBV RT-PCR Reagent is a solution for the identification of RNA from SBV using real-time RT-PCR.

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 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 the identification of the accumulating product without the need to re-open the reaction tubes afterward.

The *virotype* SBV RT-PCR Reagent contains all of the necessary reagents for the identification of SBV RNA, including a positive control. With this reagent, both reverse transcription and PCR are performed in one reaction tube, reducing the risk of contamination.

The *virotype* SBV RT-PCR reagent uses two specific primer/probe combinations: one for SBV RNA yielding FAM™ fluorescence and one for a housekeeping gene present within the sample yielding HEX™ fluorescence.

A Positive Control serves to verify the functionality of the reaction mix for the amplification of the SBV RNA target.

RNA extraction

Prior to real-time RT-PCR, viral RNA must be extracted from the starting material. QIAGEN offers a range of products for RNA extraction from animal samples.

- QIAamp® *cador*® Pathogen Mini Kit
- QIAamp Viral RNA Mini Kit
- RNeasy® Mini Kit
- RNeasy® Fibrous Tissue Mini Kit

If RT-PCR is not performed immediately after extraction, store the RNA at –70°C.

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 nucleic acids.
- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Rotor-Gene® Q or 96-well plate real-time cycler with appropriate fluorescent channels
- Rotor-Gene Q software version 1.7.94 or higher, or appropriate software for chosen 96-well plate cycler
- Strip Tubes and Caps, 0.1 ml, for use with Rotor-Gene Q (cat. no. 981103 or 981106) or 96-well optical microplate with optical sealing film or cover for chosen 96-well plate 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 an assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test reagent past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

No template control (NTC)

At least one NTC reaction should be included in each PCR run, containing all the components of the reaction except for the pathogen template. This enables identification of contamination in the reagents.

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 SBV RT-PCR Reagent to test for successful amplification of the target.

Extraction and amplification control

For increased process safety and convenience, an extraction and amplification control assay is included in the form of a second primer/probe set that identifies a housekeeping gene present within the sample. This allows both extraction and amplification to be monitored.

Protocol: Real-time RT-PCR for identification of Schmallenberg virus RNA

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 (NTC, nuclease-free water) 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.
- Before use, spin the reagents briefly.

Procedure

1. **Pipet 20 µl of the Master Mix into each reaction tube. Then add 5 µl of the sample RNA (Table 1).**

   Include positive and no template control reactions.
   Positive control: Use 5 µl of the positive control (Positive Control) instead of sample RNA.
   No template control: Use 5 µl of nuclease-free water instead of sample RNA.

Table 1. Preparation of reaction mix

Component	Volume
Master Mix	20 µl
Sample	5 µl
Total volume	25 µl

2. **Close the reaction tubes with the corresponding caps.**
3. **Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2. Select the green and yellow channels on the Rotor-Gene Q.**

Table 2. Filter settings for reporter

Component	Reporter	Quencher
SBV	**FAM**	TAMRA™
Internal control	**HEX**/JOE™*	TAMRA
Passive reference†	ROX™	

* Use the option appropriate for your thermal cycler.

† Internal reference for use with the Applied Biosystems® ABI PRISM® Sequence Detection Systems.

4. **Run the real-time RT-PCR protocol according to Table 3 if running only the *virotype* SBV RT-PCR Reagent.**

Table 3. Real-time RT-PCR protocol for SBV

Temperature	Time	Number of cycles
45°C	10 min	1
95°C	10 min	1
95°C	15 s	
56°C*	30 s	40
72°C	30 s	

* Fluorescence data collection.

5. **Run the real-time RT-PCR protocol according to Table 4 if running other *virotype* reagent assays simultaneously (i.e., *virotype* BTV, *virotype* BVDV, *virotype* CSFV, *virotype* PRRSV NA/EU, and/or *virotype* Influenza A).**

Table 4. Real-time RT-PCR protocol for simultaneous assays

Temperature	Time	Number of cycles
50°C	20 min	1
95°C	15 min	1
95°C	30 s	
57°C†	45 s	40
68°C	45 s	

† Fluorescence data collection.

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 channel with a C_T* <35. The NTC must not give a fluorescence signal.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 5 on page 17.

SBV has been identified in the sample, and the result is valid, if the following criteria are met:

- The sample yields a signal in both the FAM and HEX† channel.
- The Positive Control yields a signal in both the FAM and HEX channel.
- The NTC yields no signal.

Note that very high concentrations of SBV RNA in the sample may lead to a reduced HEX signal or no HEX signal due to competition with the internal control.

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

† Green and yellow on the Rotor-Gene Q.

SBV has not been identified in the sample, and the result is valid, if the following criteria are met:

- The sample yields a signal in only the HEX channel.
- The Positive Control yields a signal in both the FAM and HEX channel.
- The NTC yields no signal.

A HEX signal means that extraction and amplification were successful as the housekeeping gene (β-actin mRNA) within the sample is amplified.

The sample results are inconclusive, and the assay is invalid, if the following occurs:

- The sample yields no signal in the FAM and HEX channel.

If no signal is identified in both the FAM (pathogen) and the HEX (Internal Control) channel, the result is inconclusive. The absence of a signal for the housekeeping gene indicates PCR inhibition and/or other malfunctions.

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

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 reaction mix or incorrect cycling conditions.

Table 5. Results interpretation table*

	Reporter	
Sample result	**FAM (pathogen)**	**HEX (IC)**
SBV positive	X	X
SBV positive (strong positive)	X	
SBV negative		X
Inconclusive result		

* 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 channel. The negative control must yield no signal in the FAM and HEX channel. For a complete explanation of possible sample results please refer to "Data Analysis and Interpretation" on page 15.

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
virotype SBV RT-PCR Reagent (96)	For 96 reactions: Master Mix, Positive Control	281615
virotype SBV RT-PCR Reagent (480)*	For 480 reactions: Master Mix, Positive Control	281617
Related products		
virotype BVDV RT-PCR Reagent (96)†	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control	280385
virotype BTV RT-PCR Reagent (96)†	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control	280475
virotype ASFV PCR Reagent (96)	For 96 reactions: Master Mix, Positive Control	281915
virotype CSFV RT-PCR Reagent (96)†	For 96 reactions: Master Mix, Positive Control	281815
virotype Influenza A RT-PCR Rgt (96)†	For 96 reactions: Master Mix, Positive Control	282615

* Available only on request.
† Other reagent sizes available on request; see www.qiagen.com.

Product	Contents	Cat. no.
virotype PEDV/TGEV RT-PCR Rgt (96)*	For 96 reactions: Master Mix, Positive Control	283615
virotype PRRSV NA/EU RT-PCR Rgt (96)*	For 96 reactions: Master Mix, Positive Control	282315
bactotype® MAP PCR Reagent (96)*	For 96 reactions: Master Mix, Internal Control DNA, Positive Control	285915
bactotype Mg/Ms PCR Reagent (96)*	For 96 reactions: Master Mix, Positive Control	288115
QIAamp *cador* Pathogen Mini Kit (50)†	For 50 preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers	54104
QIAamp Viral RNA Mini Kit (50)†	For 50 RNA preps: 50 QIAamp Mini Spin Columns, carrier RNA, Collection Tubes (2 ml), RNase-free buffers	52904
RNeasy Mini Kit (50)†	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104

* Other reagent sizes available on request; see www.qiagen.com.
† Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
RNeasy Fibrous Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers	74704
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor	9001570

QIAGEN offers a range of real-time PCR and real-time RT-PCR reagents for the identification of RNA and DNA from animal pathogens. Visit www.qiagen.com/Animal-pathogens-and-genotyping for more information about the *bactotype*, *cador*, and *virotype* products.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

The purchase of this product allows the purchaser to use it for amplification and identification of nucleic acid sequences for laboratory use only. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

Limited License Agreement for *virotype* SBV RT-PCR Reagent.

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.

2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.

3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

www.qiagen.com

Canada ■ techservice-ca@qiagen.com

USA ■ techservice-us@qiagen.com

1075542 02/2015

Sample & Assay Technologies