

ID Gene™ Schmallenberg Duplex

Ref: IDSBV-50 / IDSBV-100

50 / 100 tests



Real-time RT-PCR for the qualitative detection of Schmallenberg virus
Suitable samples: ruminant blood, serum or encephalon

In-vitro Use

General information

▪ Characteristics

ID Gene™ Schmallenberg Duplex (IDSBV) kit is a real-time RT-PCR kit that amplifies a target sequence in Schmallenberg (SBV) viral genome.

This kit is a **qualitative duplex test**. It simultaneously amplifies target RNA and endogenous internal control.

The kit contains the target positive control (TPC-SBV) which is to be extracted in the same manner as the samples to validate the extraction and amplification of the target.

This kit can be used to test ruminant whole blood collected in EDTA, serum or encephalon.

▪ Kit composition and storage conditions

The IDSBV kit contains the reagents shown below:

<i>Reference</i>	<i>Component</i>	<i>Volume</i>	<i>Description</i>
<i>TPC-SBV</i>	Target Positive control	550 µl 1 vial	Inactivated SBV, diluted in a virus-negative serum matrix, freeze-dried and calibrated at between 10 and 100 times the detection limit of the method (MDL). Freeze-dried pellet to be reconstituted in 550 µl distilled or Nuclease-free water.
<i>ARM-SBV</i>	Amplification Reaction Mix	400 µl 1 / 2 tubes (white cap)	Ready-to-use reaction mixture containing the reverse transcriptase, Taq polymerase and oligonucleotides for detection of SBV and of the endogenous non-target positive control.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) such as not to thaw components more than 3 times.

▪ Required equipment, not provided in the kit

All material used should be of suitable quality for molecular biology.

Amplification Instrument:

Real-time thermal cycler capable of reading the following wavelengths: 525 nm (FAM), 548 nm (Yakima Yellow, equivalent to VIC) and 650 nm (Cy5).

Examples of compatible thermal cyclers: CFX96, Chromo4 Biorad, LC480 I, LC480 II, LC96 Roche, 7500 AB, and Rotorgen Qiagen. Please contact us for use with other thermal cyclers.

Consumables:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl with Nuclease-free filtered tips
- 1,5 ml tubes
- 96-well PCR plates, strips or PCR micro-tubes (that have an optical quality compatible with the thermal cycler) and appropriate adapted adhesive film or caps

Reagents:

- Distilled or Nuclease-free water;

Remarks and precautions

The material used contains less than 0,1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Extraction and amplification controls

▪ Positive controls

The IDSBV kit contains the following positive controls:

- Target Positive Control (TPC-SBV):

This control consists of inactivated SBV diluted in a virus-negative serum matrix, calibrated between 10 to 100 times the detection limit of the method (MDL).

This control validates the extraction and amplification of the target.

This control is prepared and extracted in the same way as samples.

- Endogenous Non-Target Positive Control (NTPCen):

This control is constitutively present in the cells of the test sample. It validates cell lysis and amplification of a non-target gene. It also confirms the presence of cells, and gives an indication of the state of the sample.

▪ Negative controls

It is recommended to include the following negative controls:

- Negative extraction control (NEC)

It is recommended to include a negative control for extraction (NEC) in each run.

This control is to be prepared and extracted in the same way as samples, but does not contain any target RNA. The volume occupied by the sample is replaced by a non-reactive matrix or Nuclease-free water.

- Negative control for amplification (NAC)

This control contains 8 µl of reaction mix (ARM-SBV) and 5 µl of Nuclease-free water. It is included in each analysis cycle to control for the presence of any aerosol contaminants.

Amplification protocol

▪ Extraction of viral RNA

The viral RNA must be extracted from the sample before being amplified by RT-PCR.

For this, IDvet Genetics offers a range of extraction kits that conform to the French NF U47-600 standard:

Description	Product name	Product code
Magnetic bead extraction system	ID Gene™ Mag Universal Extraction Kit	MAG192/MAG384
	ID Gene™ Mag Fast Extraction Kit	MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250

▪ Extraction of the controls

The volume of the control to extract is described in the table below:

Important:

- The volume indicated is valid regardless of the extraction system.
- The control must be extracted at the same time as the samples.

Control	Volume
TPC-SBV	50 µl

Note: If the NEC is prepared with a negative matrix sample, refer to the extraction kit protocol for the matrix in question.

▪ Preparation of the real-time PCR (RT-PCR) amplification reaction

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (TPC-SBV) from the other samples.
2. Thaw the IDSBV kit, ideally at +5°C (+/- 3°C) in a refrigerated rack. Thaw at room temperature (+21°C ± 5°C) only if the mix is to be used immediately after thawing.
3. Homogenise the contents of the **ARM-SBV** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-SBV** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add :
 - 5 µl of RNA extracted from each sample to be analyzed
 - 5 µl of RNA extracted from the TPC-SBV
 - 5 µl of extracted NEC
 - 5 µl of Nuclease-free water (NAC)
6. Cover the plate or strips with appropriate adhesive film or caps.

▪ Programming the amplification phase

1. Program the thermal cycler detectors to read the following wavelengths for each well:

Target	Fluorophore	λ (nm)	Quencher
SBV	FAM	495-525	non fluorescent
NTPCen	VIC / Yakima Yellow	426-548	non fluorescent (compatible VIC/HEX)

Note: For devices requiring an internal reference, the amplification mix IDSBV contains ROX.

2. Choose between the two 2 different amplification programs validated by IDvet Genetics:
 - Standard program (allows for PCR kits from different vendors to be used in a single session) or
 - Rapid program

Step	Standard program	Rapid program	Number of cycles
(1) Reverse transcription	10 min at 45°C	10 min at 45°C	1
(2) Polymerase activation	10 min at 95°C	2 min at 95°C	1
(3) RNA denaturation/elongation	15 sec at 95°C	10 sec at 95°C	40
	60 sec at 60°C	30 sec at 60°C	

Note: The fluorescence reading is taken at the end of the elongation phase at 60°C.

3. Enter one or these programs in the thermal cycler and select a final volume of **13 µl per PCR**. If different volumes are combined in a single session, enter the largest volume on the plate.
4. Place the PCR plate, PCR strips or capillaries in the thermal cycler and start the program.

Validation and interpretation of results

▪ Assay validation

The analysis of results is based on the Cq value of each sample that is obtained by each detector.

The test is validated according to criteria outlined in the table below. **The result cannot be reliably interpreted if any of the criteria are not met.**

Control	Expected result	Acceptability criteria
<i>TPC-SBV</i>	Detected in FAM	Refer to the Cq value given in the quality control certificate
<i>NTPCen</i>	Detected in VIC in each sample	Presence of a characteristic curve
<i>NEC</i>	Nothing detected if water used	Complete absence of a characteristic curve
	Detected in VIC if virus-negative sample used	Presence of a characteristic curve
<i>NAC</i>	No detection	Complete absence of a characteristic curve

▪ Suggested interpretation of results

For each sample, results may be interpreted according to the following criteria:

Sample	SBV signal	NTPC-SBV signal	Interpretation
Individual	Detected	Detected or Not detected	Animal detected for SBV
	Not detected	Detected	Animal not detected for SBV
	Not detected	Not detected	A problem occurred during sample distribution or extraction process / PCR reaction was inhibited

Note: TPC-SBV could be used as "sentinel" because it is a positive sample calibrated at the limit of detection method.

Non-validated samples:

- If the NTPCen is not detected but the sample is detected positive for SBV, consider the sample as positive.

- If the NTPCen is not detected:

- A problem occurred during sample distribution or during the extraction process. In this case, the sample is to be extracted again.
- Or the PCR reaction was inhibited. In this case, perform a new amplification run following the procedure below.

Procedure to follow if the PCR reaction was inhibited:

1. Dilute the extracted RNA 10-fold in Nuclease-free water.
2. Repeat the amplification phase on 5 µl of this dilution.
3. If the NTPCen is detected, interpret the sample according to table above.
4. If the NTPCen is not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

For questions or technical support, please contact: support.genetics@id-vet.com

For additional information, visit www.id-vet.com