Certified
management
system

ID Gene™ BVD/BD Triplex

Ref: IDBVD-50 / IDBVD-100

50 / 100 tests

Real-time RT-PCR for the qualitative detection
of Bovine Viral Diarrhea and Border Disease viruses
Suitable samples: ruminant blood, serum, milk, spleen and ear notch samples
(Individual samples or pools up to 100)

***In-vitro* Use**

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General information

Characteristics

ID Gene™ BVD/BD Triplex (IDBVD) kit is a real-time RT-PCR kit that amplifies a target RNA sequence in the Bovine viral Diarrhea (BVD) or Border Disease (BD) viral genome.

This kit is a qualitative triplex test. It simultaneously amplifies target RNA, endogenous and exogenous internal controls.

The kit contains the target positive control (TPC-BVD), the target positive for ear notch sample (TPC-EN-BVD) and the non-target positive control (NTPC-BVD) which are to be extracted in the same manner as the samples to evaluate extraction efficiency and to detect the presence of PCR inhibitors.

This kit can be used to test ruminant whole blood collected in EDTA or serum. It may also be used on viral RNA extracted from cell samples such as milk, organs (*e.g,* spleen) or ear notch samples. Both individual samples and pools up to 100 may be tested.

Kit composition and storage conditions

The IDBVD kit contains the reagents listed below:

Reference	*Component*	*Volume*	*Description*
TPC-BVD	Target Positive control	550 µl 1 vial	BVDV-positive whole blood sample, freeze-dried and calibrated between 10 and 100 times the method detection limit (MDL). Freeze-dried pellet to be reconstituted in **550 µl** distilled or Nuclease-free water.
TPC-EN-BVD	Target Positive Control for Ear Notch sample	550 µl 1 vial	Homogenate of BVDV-positive ear notch sample, freeze-dried and calibrated between 10 and 100 times the method detection limit (MDL). Freeze-dried pellet to be reconstituted in **550 µl** distilled or Nuclease-free water.
NTPC-BVD	Non-Target Positive Control	2200 µl 1 vial	Non-pathogenic inactivated viral strain. Freeze-dried pellet to be reconstituted in **2200 µl** distilled or Nuclease-free water.
ARM-BVD	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mixture containing the reverse transcriptase, Taq polymerase and oligonucleotides for amplification and detection of BVD/BD viruses, the endogenous and exogenous non-target positive controls.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid multiple freeze/thaw cycles (> 3 not recommended).

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 Rotor-Gene Q Qiagen. Please contact us regarding suitability with other thermal cyclers

Consumables:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl
- 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 IDBVD kit contains the following positive controls:

- Exogenous Non-Target Positive Control (NTPC-BVD) :

The exogenous positive control is a non-pathogenic inactivated RNA virus.
This control allows to evaluate the efficiency of the extraction process and to detect the presence of inhibitors in the amplification step.
This control is to be added to every sample as well as to the other controls (TPC-BVD and NEC) before extraction.

- Target Positive Control (TPC-BVD) and Target Positive Control for Ear Notch sample (TPC-EN-BVD):

These controls consist of BVDV-positive samples, calibrated between 10 and 100 times the method detection limit (MDL).
These controls validate the extraction and amplification of the target.
These controls are 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. Its function is to validate (1) cell lysis and (2) amplification of a non-target gene. It also confirms the presence of cells, and gives an indication of the quality of the sample.

▪ Negative controls

It is recommended to include the following negative controls in each run:

- Negative extraction control (NEC)

This control should be prepared and extracted in the same way as samples, but should 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-BVD) 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 ID Gene™ Mag Fast Extraction Kit	MAG192/MAG384 MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250
Direct lysis system for ear notch samples	ID Gene™ Direct lysis of Ear Notch Samples	DLB

▪ Extraction of the controls

The volumes of the control to extract are described in the table below:

Important:

- The volumes indicated are valid regardless of the extraction system.
- The controls must be extracted at the same time as the samples.

Control	*Volume*
TPC-BVD	**50 µl**
NTPC-BVD to add to TPC-BVD, TPC-EN-BVD, NEC and to each sample	**5 µl** for magnetic beads or column extraction **20 µl** for direct lysis

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

▪ 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-BVD) from the other samples.
2. Thaw the IDBVD 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-BVD** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-BVD** 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-BVD
   - 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*
BVD	FAM	495-525	non fluorescent
NTPC	Cy5	649	non fluorescent
NTPCen	VIC / Yakima Yellow	426-548	non fluorescent (compatible VIC/HEX)

Note: For devices requiring an internal reference for optical calibration, the amplification mix ARM-BVD 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 run) 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 60 sec at 60°C	10 sec at 95°C 30 sec at 60°C	40

Note: The fluorescence is read 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 run, 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 (Quantification cycle) value of each sample that is obtained by each detector. The Cq is also known as the Ct value (Threshold cycle).

The test is validated according to criteria outlined in the table below. **Results should not be interpreted if any of these criteria are not met.**

Control	*Expected result*	*Acceptability criteria*
TPC-BVD *TPC-EN-BVD*	Detected in FAM	Refer to the Cq value given in the quality control certificate
NTPC-BVD	Detected in Cy5 in each sample	+ 3 Cq of control reported to Cq of NEC
NTPCen	Detected in VIC in each sample	Presence of a characteristic curve
NEC	Detected in Cy5 if water used	+ 3 Cq of control with respect to the Cq value indicated in the quality control certificate
	Detected in Cy5 and VIC if virus-negative sample used	Presence of a characteristic curve
NAC	No detection	Complete absence of a characteristic curve

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

- **Suggested interpretation of results**

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

Sample	*BVD signal*	*NTPC-BVD signal*	*Interpretation*
Individual	Detected	Detected or Not detected	Animal positive for BVD or BD; Confirm IPI status by testing the sample after 3 weeks
	Not detected	Not detected or $Cq_{NTPC-BVD} > Cq_{NEC} + 3$	PCR reaction was inhibited
	Not detected	Detected	Animal negative for BVD or BD
Pooled	Detected	Detected or Not detected	At least 1 animal positive for BVD or BD Individual sample analysis is required
	Not detected	Not detected or $Cq_{NTPC-BVD} > Cq_{NEC} + 3$	PCR reaction was inhibited
	Not detected	Detected	Mix of negative samples

Non-validated samples:

- **If the NTPC-BVD is not detected but the sample is detected positive for BVD, 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.
- **If $Cq_{NTPC-BVD} > Cq_{NEC} + 3$ and no signal is detected for BVD**, the reaction is 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 10times in Nuclease-free water.
2. Repeat the amplification reaction on 5 µl of this dilution.
3. If the NTPC is detected, interpret the sample according to table above.
4. If the NTPC is not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

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

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