INSTRUCTIONS FOR USE

VetMAX™ BVDV 4ALL Kit

TaqMan® real-time RT-PCR for the detection of the BVD virus (type 1, 2 and 3) and the Border Disease virus (type 1 to 6)

Catalog Number BVD4ALL
Doc. Part No. 100021232 Pub. No. MAN0008959 Rev. B.0

Technology Species Nucleic acid isolated from matrices Test type
Real-time RT-PCR (RNA)
• Duplex assay
• Endogenous or exogenous [for serum only] IPC
Bovine Sheep Goat Wild ruminants Blood Serum Ear biopsies Individual or pooled [according to the type of specimen and the purification protocol used]

⚠️ 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™ BVDV 4ALL Kit was developed for detection of a sequence from the 5’UTR region of the viral genome (RNA) of the BVD (Bovine Viral Diarrhea) and BD (Border Disease) viruses. It is applicable to all ruminants: bovine, small ruminants and wild ruminants. It can be used on viral RNA extracted from serum, whole blood collected in EDTA tubes and ear biopsies. For ear biopsies, it is possible to extract viral RNA by the rapid lysis method [for example, with the VetMAX™ Ear Notch Fast Lysis Kit (Cat. No. FLK)]. Complete protocols for viral RNA extraction from these matrices are available upon request from Technical Support. Depending on the viral RNA isolation protocol used as well as the specimen type, the VetMAX™ BVDV 4ALL Kit can be used on individual or pooled samples (according to national, regional or local regulations). Each RNA sample obtained after extraction or rapid lysis is analyzed in a single well: the same well is used for specific detection of the viral RNA of the BVD and BD viruses and for detection of an IPC (Internal Positive Control). A positive IPC signifies both successful extraction and the absence of PCR inhibitors in the sample. The IPC target is endogenous to cellular samples (blood and ear notches). Serum samples require addition of exogenous IPC (provided with the kit) before extraction of viral RNA.

Kit contents and storage
On receipt, the entire kit should be stored between −30°C and −10°C. When using the kit for the first time, follow the storage recommendations in the following table for each component:

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Volume (100 25-μL reactions)</th>
<th>Storage Upon receipt</th>
<th>After initial use</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 - Mix BVDV 4ALL (Green tube)</td>
<td>Mix for TaqMan® RT-PCR. Contains: • The detection system for BVDV 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™. • The buffer, the reverse transcriptase and the PCR enzyme.</td>
<td>4 × 500 μL</td>
<td>−30°C to −10°C</td>
<td>−30°C to −10°C</td>
</tr>
<tr>
<td>4c - EPC BVDV 4ALL (Blue tube)</td>
<td>External Positive Control: BVDV positive control. This is an inactive biological agent, to be extracted or lysed and then amplified during real-time RT-PCR.</td>
<td>150 μL</td>
<td>−30°C to −10°C</td>
<td>−30°C to −10°C</td>
</tr>
<tr>
<td>5 - IPC BVDV 4ALL[1] (Yellow tube)</td>
<td>Internal Positive Control (for serum only): Exogenous internal control, to be added to the lysis solution used for RNA extraction from serum samples and positive and negative extraction controls. It can also be used as an amplification control only, when added to extracted RNA.</td>
<td>500 μL</td>
<td>−30°C to −10°C</td>
<td>−30°C to −10°C</td>
</tr>
</tbody>
</table>

[1] For small extraction series, it is recommended to aliquot 5-IPC BVDV 4ALL in a minimum volume of 50 μL to avoid more than 3 freeze/thaw cycles.
Extraction and amplification controls

The VetMAX™ BVDV 4ALL Kit contains 2 controls, enabling validation of the extraction and amplification of viral RNA.

4c - EPC BVDV 4ALL: BVDV positive control

Extract or lyse 4c - EPC BVDV 4ALL at the same time as the samples, as described in the following table.

<table>
<thead>
<tr>
<th>Extraction or lysis method</th>
<th>Use this volume of 4c - EPC BVDV 4ALL</th>
<th>Use this elution volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica columns or magnetic beads</td>
<td>50 µL</td>
<td>50–80 µL</td>
</tr>
<tr>
<td>Fast lysis</td>
<td>50 µL</td>
<td>—</td>
</tr>
</tbody>
</table>

Store the RNA extracted from 4c - EPC BVDV 4ALL below −16°C. Store in smaller aliquots if necessary to avoid more than 3 freeze-thaw cycles.

A positive result within the specified Ct range is used to validate the extraction and the amplification of the BVDV target by real-time RT-PCR.

5 - IPC BVDV 4ALL: exogenous Internal Positive Control (for serum only)

Serum samples require an exogenous IPC source; this component is added to the lysis solution used for RNA extraction from serum samples, as well as in preparation of the BVDV positive control sample and the negative extraction control sample (NCS). The IPC target is already present in cellular samples (blood and ear notches), so addition of 5 - IPC BVDV 4ALL is not required for these samples.

For extraction series that only include serum samples, add 5 µL of 5 - IPC BVDV 4ALL to the lysis buffer during RNA extraction of the samples, BVDV positive control, and NCS. Alternatively, for use as an amplification control only, add 5 µL of 5 - IPC BVDV 4ALL to 50 µL of extracted RNA.

For extraction series that do not include serum samples, prepare lysis solution without 5 - IPC BVDV 4ALL for the cellular samples, the BVDV positive control, and NCS.

For extraction series that include both serum and cellular samples:

- Add 5 µL of 5 - IPC BVDV 4ALL to the lysis buffer for the serum samples, the BVDV positive control, and NCS.
- Prepare lysis solution without 5 - IPC BVDV 4ALL for the cellular samples in the extraction series.

Refer to the Ct values for the IPC target in “Validation” and “Interpretation of results” for criteria for validation of samples or controls.

We recommend including two negative controls to confirm correct analysis:

NCS: negative extraction control

This control consists of reagents 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) that undergoes the same treatment as the samples.

This control permits validation of the absence of contamination during the extraction and the real-time RT-PCR reactions. Refer to the Ct values for the NCS in “Validation” for criteria validating non-contamination.

NC: negative amplification control

This is the amplification mix deposited on the plate during the preparation of the real-time RT-PCR, with 5 µL of DNase/RNase-free water added to adjust the reaction to 25 µL or 15 µL depending on the application type (depends on the viral RNA extraction method). For all sample matrices, this control confirms the absence of contamination during real-time RT-PCR reaction preparation if it is negative for all targets (BVDV and IPC).

Materials required but not provided

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

- Adjustable 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 thermal cycler capable of detecting the following fluorophores: FAM™ (emission maximum: λ515 nm); VIC™ (emission maximum: λ554 nm); and ROX™ passive reference
- Optical-quality consumables compatible with the thermal cycler used:
  - PCR plates with 96 wells, PCR strips (8 or 12 wells), microtubes or capillaries
  - Films or compatible caps

Analysis procedure

The real-time RT-PCR reaction volume is 25 µL or 15 µL depending on the application type (depends on the viral RNA extraction method used):
**Extraction of viral RNA**

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

Before using this kit for the first time, prepare a BVDV positive control sample by extracting 4c - EPC BVD4 ALL using the same method as for the test samples. The BVDV positive control sample can be used in subsequent RT-PCRs with test samples that have been extracted with the same method.

For serum samples, add 5 µL of 5 - IPC BVDV 4ALL to the lysis solution used for RNA extraction from the samples, BVDV positive control, and NCS.

**NOTE:** For information about extraction methods that are compatible with and validated for the VetMAX™ BVDV 4ALL Kit, as well as information on the VetMAX™ Ear Notch Fast Lysis Kit (Cat. No. FLK), please contact Technical Support.

**Preparation of the real-time RT-PCR**

1. Create an analysis plan for distribution of the mixes and samples. Keep the BVDV positive control away from the other samples if possible.
2. Thaw 3 - Mix BVDV 4ALL at +2°C to +8°C, on ice or on a refrigerated rack.
3. Mix 3 - Mix BVDV 4ALL by gentle agitation, then briefly centrifuge.
4. Depending on the application type, add 20 µL or 10 µL of 3 - Mix BVDV 4ALL to each PCR plate well, PCR strip or capillary used.
5. Add RNA from samples and controls to the real-time RT-PCR mix solution according to the pre-set analysis plan:

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Component</th>
<th>Sample volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample for analysis</td>
<td>RNA extracted from sample</td>
<td>5 µL</td>
</tr>
<tr>
<td>BVDV positive control</td>
<td>RNA extracted from 4c - EPC BVDV 4ALL</td>
<td>5 µL</td>
</tr>
<tr>
<td>Negative extraction control (NCS)</td>
<td>Negative extraction control sample</td>
<td>5 µL</td>
</tr>
<tr>
<td>Negative amplification control (NC)</td>
<td>DNase/RNase-free water</td>
<td>5 µL</td>
</tr>
</tbody>
</table>

6. Cover the PCR plate, PCR strips or capillaries with an adhesive plate cover or compatible caps.

**Amplification by real-time RT-PCR**

1. Following the manufacturer’s instructions set up the following parameters for the real-time RT-PCR run.
   - Reaction volume: 15 µL (rapid lysis application) or 25 µL (standard application)
   - ROX™ passive reference dye: included in 3 - Mix BVDV 4ALL
2. Set up and assign TaqMan® probe reporter dyes and quenchers for each well used in the analysis:

<table>
<thead>
<tr>
<th>Target</th>
<th>Reporter</th>
<th>Quencher</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV</td>
<td>FAM™ dye</td>
<td>NFQ [Non-Fluorescent Quencher]</td>
</tr>
<tr>
<td>IPC</td>
<td>VIC™ dye</td>
<td>TAMRA™ dye(1)</td>
</tr>
</tbody>
</table>

Passive reference: ROX™ dye(2)

(1) TAMRA™ fluorophore must be set up for real-time RT-PCR analysis if the thermal cycler is capable of detecting it. For other thermal cyclers, lack of detection of this fluorophore does not affect the accuracy of the reading.
(2) ROX™ passive reference must be set up if the thermal cycler does not set it up automatically.
3. Create the following RT-PCR program for the analysis:

<table>
<thead>
<tr>
<th>Program 1</th>
<th>Step repetitions</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>1×</td>
<td>45°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Step 2</td>
<td>1×</td>
<td>95°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Step 3</td>
<td>45×</td>
<td>95°C</td>
<td>15 seconds</td>
</tr>
</tbody>
</table>

(1) Collect 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 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 RT-PCR.
2. For each detector, interpret the results according to the sample Cₜ values obtained as recommended below.
Validation
To validate the real-time RT-PCR run, refer to the C_{t} QC values listed in the Certificate of Analysis of the batch used for the test. The run is validated if the following criteria are met:

<table>
<thead>
<tr>
<th>Control reaction</th>
<th>BVDV target (FAM™ dye)</th>
<th>IPC target (VIC™ dye)</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No 5 - IPC BVDV 4ALL(1)</td>
<td>With 5 - IPC BVDV 4ALL(2)</td>
</tr>
<tr>
<td>BVDV positive control</td>
<td>C_{t} = C_{t} QC - BVDV of 4c - EPC BVDV 4ALL ±3C(3)</td>
<td>C_{t} &lt; 45 or C_{t} &gt; 45</td>
<td>C_{t} = C_{t} QC - IPC of 5 - IPC BVDV 4ALL ±3C(3)</td>
</tr>
<tr>
<td>NCS</td>
<td>C_{t} &gt; 45</td>
<td>C_{t} &gt; 45</td>
<td>C_{t} = C_{t} QC - IPC of 5 - IPC BVDV 4ALL ±3C(3)</td>
</tr>
<tr>
<td>NC</td>
<td>C_{t} &gt; 45</td>
<td>C_{t} &gt; 45</td>
<td>C_{t} &gt; 45</td>
</tr>
</tbody>
</table>

(1) For extraction series that do not include serum samples.
(2) For extraction series that include serum samples only or a mix of serum and cellular samples.
(3) Refer to the values listed for the extraction method used in section 2.1 “EPC” of the Certificate of Analysis.
(4) The IPC value of the BVDV positive control is not used for test validation in the case of cellular samples.
(5) Refer to the values shown in Section 2.2 “IPC” of the Certificate of Analysis. Interpretation of results

Interpretation of results
For each sample analyzed, interpret the results as shown in the following tables.

<table>
<thead>
<tr>
<th>BVDV target (FAM™ dye)</th>
<th>IPC target (VIC™ dye)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{t} &lt; 45</td>
<td>C_{t} &lt; 45 or C_{t} &gt; 45</td>
<td>PCR reagents validated</td>
</tr>
<tr>
<td>C_{t} &gt; 45</td>
<td>C_{t} &lt; 45</td>
<td>BVDV detected</td>
</tr>
<tr>
<td>C_{t} &gt; 45</td>
<td>C_{t} &gt; 45</td>
<td>Invalid results(5)</td>
</tr>
</tbody>
</table>

(1) The result is invalid due to a non-compliant IPC result.

How to handle samples with invalid results
1. Dilute RNA 1:10 dilution in 1X TE buffer.
2. Perform RT-PCR analysis on 5 µL of the diluted sample.
3. If the diluted RNA is positive or negative for BVDV with a compliant IPC result, the obtained result is valid.
4. If the diluted RNA is negative for BVDV with a non-compliant IPC result, the obtained result is still invalid. In this case, proceed as follow:
   a. For ear notch, repeat the RNA extraction and RT-PCR on either a new ear notch or another sample matrix from the same animal, if allowed by national, regional or local regulations.
   b. For the other sample matrices, repeat the RNA extraction using the same sample diluted 1:10 in 1X PBS buffer and perform RT-PCR. If the result is still invalid, repeat the RNA extraction and RT-PCR on a new sample.

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- 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
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