Bovine Virus Diarrhea RNA Test Kit

VetMAX™-Gold BVDV PI Detection Kit

Catalog Number 4413938

Pub. No. 4425603 **Rev.** G

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**.

Product description

Name, intended use, and principle of the procedure

The Applied Biosystems™ Bovine Virus Diarrhea RNA Test Kit (VetMAX™-Gold BVDV PI Detection Kit, Cat. No. 4413938) is intended for use in the rapid, *in vitro* detection of bovine virus diarrhea virus (BVDV) RNA extracted from ear punch samples obtained from acutely and persistently infected (PI) cattle.

The VetMAX™-Gold BVDV PI Detection Kit can be used to identify PI animals in mixed populations of uninfected, acute, and PI cattle. Tests that produce a presumptive BVDV-PI animal result should be followed by collecting and testing a second sample from the subject animal at least three weeks after the first sample was taken. A presumptive PI animal can be ensured PI if the second test is positive.

The VetMAX™-Gold BVDV PI Detection Kit can be used to test pools of up to 24 samples for the presence of BVDV. Pool size should be determined by the testing laboratory, based on the prevalence of BVDV disease in the area from which the samples were collected. Pooled samples yielding a positive result should then be tested individually to determine the infection status of each animal in the positive pool.

BVDV is an approximately 12 kb, single-stranded, positive-RNA enveloped virus in the genus *Pestivirus* of the Flaviviridae family. BVDV infection can cause respiratory disease, enteritis, still-birth, abortion, and mucosal disease in cattle. In utero BVDV infection can induce immunotolerance, causing lifelong persistent infection (PI). PI animals continuously shed the virus, and they are the main source of BVDV infection in herds. Rapid detection of PI cattle is essential for BVDV control.

The assay is a single-well, real-time reverse transcription PCR (real-time RT-PCR) in which RNA is reverse-transcribed into cDNA, and BVDV RNA and Xeno™ RNA Control targets are amplified and detected in real time using fluorescent TaqMan® probes (hydrolysis probe chemistry). The kit contains:

- BVDV Control RNA and Xeno™ RNA Control, to serve as positive controls for the real-time RT-PCR reaction components.
- Xeno™ RNA Control, to serve as an internal positive control for the RNA purification process.
- 25X BVDV Primer Probe Mix, optimized for multiplex real-time RT-PCR amplification of the Xeno™ RNA Control and BVDV RNA targets.

Limitations

- The kit is not intended for differentiating viral subtypes.
- Samples should be handled as recommended in Table 2 on page 2 to prevent degradation of any BVDV RNA that is present.
- Prepare pooled samples from no more than 24 individual samples.
- Pooling samples may cause loss of sensitivity of detection of acutely or transiently infected (TI) animals.
- RNA purification methods should yield RNA free of RT-PCR inhibitors, which can prevent amplification of target RNA.
- Follow "Good laboratory practices for PCR and RT-PCR" on page 5 to prevent false positive amplifications due to contamination of test samples with PCR products.

Kit contents and storage conditions

Reagents for 100 25-µL real-time RT-PCR tests are supplied.

Table 1 VetMAX™-Gold BVDV PI Detection Kit

Component	Amount	Storage
2X RT-PCR Buffer	1.375 mL	–30°C to –10°C
25X RT-PCR Enzyme Mix	110 µL	
25X BVDV Primer Probe Mix	110 µL	
Xeno™ RNA Control (10,000 copies/µL)	110 µL	
25X BVDV Control RNA (10,000 copies/µL)	15 µL	
Nucleic Acid Dilution Solution	500 µL	
Nuclease-Free Water	1.75 mL	–30°C to 25°C

Required materials not supplied

Item	Source[1]
Applied Biosystems™ 7500 or 7500 Fast Real-Time PCR System (96-well), running SDS Software v1.4	Contact your local sales representative.
QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1-mL	Contact your local sales representative.
Plates or tubes appropriate for the 7500 Real-Time PCR System (96-well)	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4326659 (500 plates), 4306737 (20 plates), or equivalent
MicroAmp™ Optical Adhesive Film	4311971 (100 covers), 4360954 (25 covers), or equivalent
Plates or tubes appropriate for the 7500 Fast or QuantStudio™ 5 Real-Time PCR System (96-well)	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4366932 (200 plates), 4346906 (20 plates), or equivalent
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Optical Adhesive Film	4311971 (100 covers), 4360954 (25 covers), or equivalent
Nuclease-free pipettes and filtered pipette tips	MLS
Nuclease-free reagent tubes for preparing master mixes	MLS
1X Phosphate Buffered Saline (PBS), pH 7.4	MLS
2 ice buckets: • One for the PCR setup area where the master mix is prepared • One for the area where RNA may be present	MLS

[1] Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Purify RNA from individual or pooled samples

Table 2 Sample handling recommendations

Step or process	Recommendation
Transport/storage of samples	Approximately –80°C to –20°C (transport on dry ice).
Obtain a 3-mm circular ear punch	The ear punch can be: • Obtained directly from an animal • Punched out of an ear notch collected from an animal
Preparation of individual ear punch supernatant	Prepare the ear punch supernatants on the same day as RNA isolation. 1. Place one dry ear punch sample into a 2-mL tube, then add 200 µL of 1X PBS. 2. Vortex at high speed for 10 minutes at 19–25°C. 3. Centrifuge at 10,000 × *g* for 30 seconds. 4. Remove 50 µL of supernatant for RNA purification. **Note:** Avoid freezing and thawing ear punch supernatants, which may cause a loss of sensitivity.
(Optional) Preparation of pooled ear punch supernatants	Pool up to 24 individual ear punch supernatants (pool size determined by the testing laboratory) as follows: 1. Add 25 µL of the individual ear notch supernatant to a new centrifuge tube, for a final volume of up to 600 µL. 2. Briefly vortex the pooled supernatants to ensure sufficient mixing of samples. 3. Process pooled samples in the same way as individual samples. 50 µL of the pooled supernatant is used for each RNA purification. **Note:** Save the individual ear notch supernatants at 4°C for further testing, if required. Do not freeze the supernatants.
Preparation of mock-purified samples (for use in extraction control PCRs)	Prepare duplicate mock-purified samples, using 1X PBS as the starting material. Process with the same RNA purification method as for test samples.
Proposed RNA purification method	MagMAX™-96 Viral RNA Isolation Kit (Cat. Nos. AM1836 and AMB1836-5), MagMAX™ Pathogen RNA/DNA Kit (Cat. No. 4462359).
Required modifications to RNA purification method	• Add 0.8 µL of undiluted Xeno™ RNA Control (8,000 copies) per purification to the lysis solution used for RNA purification. • Add carrier RNA to the lysis solution according to the manufacturer's recommendation. Add carrier RNA in addition to Xeno™ RNA Control. Carrier RNA is included in the MagMAX™-96 Viral RNA Isolation Kit (Cat. Nos. AM1836 and AMB1836-5) and MagMAX™ Pathogen RNA/DNA Kit (Cat. No. 4462359).

Perform real-time RT-PCR

1 Plan the reactions and thaw the reagents

a. Plan the reactions: On each plate, include the following control reactions (see "Add sample RNA, controls, or Nuclease-Free Water to each reaction well" on page 3).
- Positive control (prepare duplicate reactions); use 10,000 copies each of BVDV Control RNA and Xeno™ RNA Control per reaction.
- No-template control (NTC) (prepare duplicate reactions); use Nuclease-Free Water in place of sample RNA.
- Extraction controls; use the mock-purified samples (two samples).

b. Thaw all reagents on ice, gently vortex each tube to mix the contents thoroughly, then briefly centrifuge to collect the solution at the bottom of the tube. Keep the reagents on ice.

2 Prepare the RT-PCR master mix on ice

Include 10% overage to ensure that you have enough RT-PCR master mix for all the samples.

Component	Volume per 25 µL reaction
2X RT-PCR Buffer	12.5 µL
25X BVDV Primer Probe Mix	1.0 µL
25X RT-PCR Enzyme Mix	1.0 µL
Nuclease-Free Water	2.5 µL
Total volume of RT-PCR master mix	**17.0 µL**

3 Dispense the master mix

Dispense 17 µL of RT-PCR master mix to the wells of a PCR plate on ice.

4 Add sample RNA, controls, or Nuclease-Free Water to each reaction well

a. Add each component according to the following table:

Reaction type	Component	Volume per reaction
Test sample	Sample RNA	8.0 µL
NTC	Nuclease-Free Water	8.0 µL
Extraction control	Mock-purified sample	8.0 µL
Positive control	25X BVDV Control RNA	1.0 µL
	Xeno™ RNA Control	1.0 µL
	Nucleic Acid Dilution Solution	6.0 µL

b. Seal each reaction vessel, mix, then centrifuge briefly to bring the contents to the bottom.

5 Set up and run the real-time PCR instrument

For detailed information to set up and run the instrument, see the appropriate documentation for your instrument.

a. Following the manufacturer's instructions, set up the run using the following parameters:
- Experiment type: Standard curve
- Run mode: Standard
- Reaction volume: 25 µL
- ROX™ passive reference dye: Included in the RT-PCR Buffer
- TaqMan® probe reporter dyes and quenchers:

Target	Reporter	Quencher
BVDV RNA	FAM™ dye	BHQ™-1 dye
Xeno™ RNA Control	CAL Fluor™ Orange 560 dye[1]	BHQ™-1 dye

[1] CAL Fluor™ Orange 560 dye has an absorbance maximum of 538 nm and an emission maximum of 559 nm. When using an Applied Biosystems™ Real-Time PCR System, calibrate with CAL Fluor™ Orange 560 dye. If that is not possible, use the VIC™ dye detector.

b. Run the thermal cycler program and collect real-time amplification data during stage 3. Thermal cycler settings:

Stage		Reps.	Temp.	Time
Reverse transcription	1	1	45°C	10 minutes
RT inactivation/initial denaturation	2	1	95°C	10 minutes
Amplification	3	40	95°C 60°C	15 seconds 45 seconds

Data analysis

See your real-time PCR instrument user guide for instructions on how to analyze your data, using the method below.

Table 3 Data analysis

Method	Details
Use the **Control-Based Threshold** setting for data analysis.	1. Select **Manual CT**. 2. Export ΔR_n values for the positive control samples of BVDV RNA and Xeno™ RNA Control. 3. Average the FAM™ and CAL Fluor™ Orange 560 values (separately) for the ΔR_n at cycle 40 for all replicates of the positive control reactions. 4. Set the threshold for the BVDV RNA target reactions at 2% of the average maximum fluorescence value of the BVDV amplification signal in the positive control reactions (containing approximately 10,000 copies of BVDV Control RNA and 10,000 copies of Xeno™ RNA Control per reaction). Example: If the average maximum fluorescence value for the BVDV target in the positive control reactions is 0.15, set the BVDV threshold at 0.003. 5. Repeat step 4 for the Xeno™ RNA Control target using a 10% threshold. Example: If the average maximum fluorescence value for the Xeno™ RNA Control target in the positive control reactions is 2.0, set the Xeno™ DNA Control threshold at 0.2.
Check the raw fluorescence data.	Verify that fluorescence increases seen in the normalized data are evident without mathematical data processing.

Interpretation of test results

Verify that your real-time RT-PCR run is valid before analyzing test sample results.

Table 4 Criteria for a valid real-time RT-PCR run

Reaction type	C_t value for BVDV RNA	C_t value for Xeno™ RNA Control
Positive control	<30	<32
NTC	40 (no signal)[1]	40 (no signal)[2]
Extraction control	40 (no signal)[1]	31–38

[1] The run is invalid if the C_t value for BVDV is <38. If the C_t value is 38–40, the RT-PCR may be contaminated. See "Troubleshooting" on page 5.

[2] The run is invalid if the C_t value for Xeno™ RNA Control is <38. If the C_t value is 38–40, the RT-PCR may be contaminated. See "Troubleshooting" on page 5.

Table 5 Interpretation of test results from individual samples

C_t value for BVDV RNA	C_t value for Xeno™ RNA Control	Interpretation
<38	<40 (signal detected)	**BVDV-positive sample**
⩽31	<40 (signal detected)	**Presumptive BVDV-PI animal sample** Tests that produce a presumptive BVDV-PI animal result should be followed by collecting and testing a second sample from the subject animal at least 3 weeks after the initial sample was taken. A presumptive PI animal can be confirmed PI if the second test is positive.
40 (no signal)	31–38	**BVDV-negative sample**
	>38	See "Troubleshooting" on page 5 if the C_t value for Xeno™ RNA Control is >38 in a BVDV-negative sample.
⩾38 and <40	<40 (signal detected)	**Suspect result** (see Table 7)

Table 6 Interpretation of test results from pooled samples

C_t value for BVDV RNA	C_t value for Xeno™ RNA Control	Interpretation
<40 (signal detected)	<40 (signal detected)	**BVDV-positive pool** Retest individual samples from the positive pool to determine which individual sample or samples caused the positive result. Interpret the retest results according to Table 5.
40 (no signal)	31–38	**BVDV-negative pool**
	>38	If the C_t value for Xeno™ RNA Control is >38, repeat the RNA purification on triplicate aliquots of the pooled supernatants, then repeat the real-time RT-PCR with each RNA sample.

Table 7 Assessment of suspect results

Analyze suspect RNA samples (BVDV C_t 38–40) for the presence/absence of RT-PCR inhibitors by calculating the Xeno™ RNA C_t shift: **Xeno™ RNA C_t shift = Xeno™ RNA $C_{t\ \text{SUSPECT SAMPLE}}$ – Avg. Xeno™ RNA $C_{t\ \text{MOCK-PURIFIED SAMPLES (EXTRACTION CONTROLS)}}$**	
Xeno™ RNA C_t shift ⩾1.5	**Xeno™ RNA C_t shift <1.5**
Repeat the real-time RT-PCR with 2 µL of the suspect RNA sample. (RT-PCR inhibitors may be present in the RNA.) **If BVDV C_t <38:** BVDV positive **If BVDV C_t ⩾38:** 1. Dilute the original diagnostic sample 1:4 if samples are being tested individually. Samples in pools are already diluted, so no further dilution of pooled samples is necessary. 2. Repeat the RNA purification on triplicate aliquots of the diluted sample. 3. Repeat the real-time RT-PCR with 8 µL of purified RNA from step 2. 4. Determine the number of samples with BVDV C_t <40. The standard deviation of the triplicates should be ±1 C_t. • 0 of 3: BVDV negative. • 1 of 3: Presumptive positive; confirm with secondary test method. • ⩾2 of 3: BVDV positive.	1. Repeat the RNA purification on triplicate aliquots of the original diagnostic sample. 2. Repeat the real-time RT-PCR with 8 µL of purified RNA from step 1. 3. Determine the number of samples with BVDV C_t <40. The standard deviation of the triplicates should be ±1 C_t. • 0 of 3: BVDV negative. • 1 of 3: Presumptive positive; confirm with secondary test method. • ⩾2 of 3: BVDV positive.

Troubleshooting

Observation	Possible cause	Recommended action
Positive control reaction: BVDV Control RNA—no signal Xeno™ RNA Control—no signal	The BVDV Control RNA or Xeno™ RNA Control was improperly handled, resulting in RNA degradation.	Use appropriate precautions against RNase contamination when handling the control RNAs. For example, wear clean gloves and use nuclease-free barrier pipette tips.
	The 25X RT-PCR Enzyme Mix was stored or handled improperly, and it lost activity.	Repeat the real-time RT-PCR with fresh reagents.
	The thermal cycler was not properly set up.	See "Set up and run the real-time PCR instrument" on page 3.
	The RT-PCR master mix was prepared incorrectly.	See "Prepare the RT-PCR master mix on ice" on page 3.
NTC reaction: Signal detected	PCR contamination.	• Repeat the real-time RT-PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time RT-PCR in an area separate from areas used for RNA purification and PCR product analysis.
Test samples: Xeno™ RNA Control —C_t value >38 *and* BVDV RNA—high signal	The Xeno™ RNA Control primers and probe are at limiting concentrations in the real-time RT-PCR. High levels of BVDV RNA in a sample can reduce amplification of Xeno™ RNA Control.	No or low signal from Xeno™ RNA Control is expected in a reaction that has a strong signal for BVDV RNA.
Test samples: Xeno™ RNA Control—no signal *and* BVDV RNA—C_t value >38	Poor RNA recovery. *or* The RNA samples contain RT-PCR inhibitors.	Check the C_t values of Xeno™ RNA Control in the mock-purified samples. A C_t value ≥38 indicates that Xeno™ RNA Control was omitted or that RNA recovery was poor: • Check that Xeno™ RNA Control was added to the lysis solution as described in "Purify RNA from individual or pooled samples" on page 2. • Check the recovery of carrier RNA used in RNA purification. If it is poor, troubleshoot your RNA preparation method. A C_t value of 31–38 indicates that RNA recovery is adequate; therefore the test samples may have RT-PCR inhibitors. See Table 7.
All samples: Atypical non-sigmoidal amplification curves when using the Control-Based Threshold setting	Atypical data (usually due to experimental error)	Repeat the real-time RT-PCR according to Table 7.

Explanation of symbols

The symbols present on the product label are explained in the following table.

	MANUFACTURER		CAUTION
	CATALOG NUMBER		POSITIVE CONTROL
	BATCH CODE		NEGATIVE CONTROL
	USE BY		TEMPERATURE LIMIT
	CONSULT INSTRUCTIONS FOR USE		KEEP AWAY FROM SUNLIGHT, KEEP AWAY FROM HEAT

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Documentation and support

Customer and technical support

In the United States, call 1-800-955-6288.

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Product documentation

Limited product warranty

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Revision	Date	Description
G	20 August 2018	Updated the list of compatible real-time PCR systems.
F	10 August 2017	• Changed data analysis to control-based threshold (Table 3). • Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
E	10 July 2013	Baseline for this revision history

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