SHENTEK

Bovine Virus Detection Kit

(BVDV/REO-3/PI-3/BPV-3/BAV-3)

User Guide

Version: A/0
For Research Use Only
Product No.: 1506741
Reagents for 50 Reactions

Huzhou Shenke Biotechnology Co., Ltd.

(IMPORTANT: Please read this document carefully before experiment.)

1. Product information

■ Product description

SHENTEK® Bovine Virus Detection Kit (BVDV/REO-3/PI-3/BPV-3/BAV-3) is designed for rapid and qualitative detection of five bovine-derived viruses contaminants in serum and cell bank, including Bovine Viral Diarrhea Virus (BVDV), Reovirus 3 (REO-3), Bovine Parainfluenza 3 Virus (PI-3), Bovine Parvovirus 3 (BPV-3), and Bovine Adenovirus 3 (BAV-3). The kit works together with SHENTEK® Virus DNA & RNA Extraction Kit (Product No.1506730).

Using multiplex real-time PCR technique, the assay can simultaneously detect three RNA viruses (BVDV/REO-3/PI-3) or two DNA viruses (BPV-3/BAV-3) in a single well, allowing for rapid and efficient detection of bovine-derived viral in raw materials of animal origin, cell banks, virus seed banks, unprocessed bulk, and final products in bioproducts process. The detection assay shows high sensitivity and specificity with a detection limit of 50 copies per reaction. Positive Control (can be purchased separately) and Internal Control (IC) are provided to ensure reliable results. Combined with SHENTEK® Virus DNA & RNA Extraction Kit, these kits can efficiently recover and detect bovine virus nucleic acid from complex sample matrices such as cells and serum.

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, mask and gloves.

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
5×Multiplex RT-PCR Buffer	NNB020	200 μL × 1 tube	-20℃
Multiplex RT-PCR Enzyme MIX	NNC102	100 μL × 1 tube	
Multiplex qPCR Reaction Buffer	NNB021	850 μL × 1 tube	
VIR Internal Control (IC)	NNA057	600 μL × 1 tube	
RNase-Free H_2O	NND008	1.2 mL × 1 tube	
BV RNA Primer&Probe MIX	NNC103	170 μL × 1 tube	-20℃, protect from light
BV DNA Primer&Probe MIX	NNC104	120 μL × 1 tube	
Multiplex IPC MIX	NNC105	170 μL × 1 tube	

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Applicable instruments, including but not limited to the following

- SHENTEK-96S Real-time PCR System
- LightCycler 480II Real-Time PCR System
- ABI 7500 Real-Time PCR System

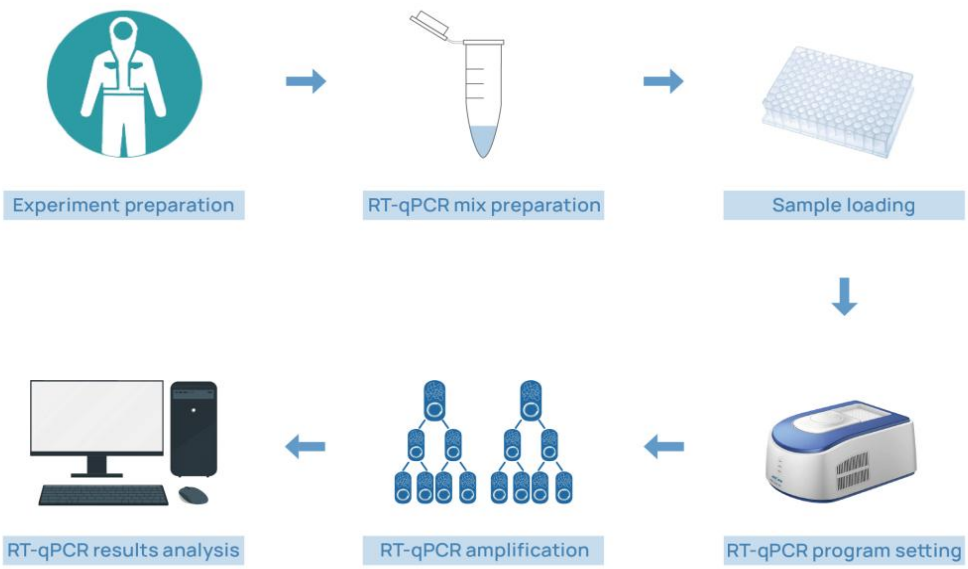
■ Required materials not included in the kit

- Sterile, RNase/DNase-free, Low Retention microcentrifuge tubes, 1.5 mL and 2.0 mL
- PCR 8-well strip tubes with caps or 96-well plates with sealing films
- Sterile, RNase/DNase-free, Low Retention tips: 1000 μL, 200 μL, 100 μL and 10 μL
- SHENTEK® Virus DNA & RNA Extraction Kit (Product No. 1506730)
- SHENTEK® BVDV, REO-3, PI-3, BPV-3, BAV-3 Positive Control (Product No. 1506736, 1506737, 1506738, 1506746, 1506739)

■ Related equipment

- Benchtop microcentrifuge
- Microplate and microtube shaker
- Vortex mixer
- Real-time PCR System
- Pipettes: 1000 µL, 200 µL, 100 µL and 10 µL

■ Workflow



2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Please handle the positive control and the extraction procedure in a biological safety cabinet and follow the BSL-2 laboratory safety guidelines.
3. Use consumables and reagents that are certified as nuclease-free.
4. Thaw the kit completely at 2-8°C or melt on ice, vortex thoroughly and centrifuge briefly.

■ Sample pretreatment

Use Virus DNA & RNA Extraction Kit for viral nucleic acid extraction.

1. Preparation of test samples

For cell matrix and bovine serum samples

(1) Collect 100-200 μL of the cell culture sample (≤10^7 cells) or serum sample, and add to a new 1.5 mL microcentrifuge tube.

(2) Add Pretreatment buffer (NND002) to the sample at a ratio of 1:10, and vortex to mix well. Incubate at room temperature (RT) for 5 minutes (min).

(3) Centrifuge at 450×g for 30 min at 4℃, carefully transfer the supernatant to a new microcentrifuge tube.

2. Preparation of control samples

Positive control samples (PCS)

(1) For the BVDV/REO-3/PI-3 assay, mix BVDV, REO-3, and PI-3 Positive Controls.

(2) For the BPV-3/BAV-3 assay, mix BPV-3 and BAV-3 Positive Controls.

(3) Follow the same extraction procedure as for test samples.

Negative control samples (NCS)

(1) Take an equal volume of RNase-Free H_2O or 1×PBS as the test sample.

(2) Follow the same extraction procedure as for test samples.

3. Samples digestion

(1) Add 100 μL Proteinase K buffer (NND025) and 50 μL Proteinase K (NND023) to each sample.

(2) Add 10 μL VIR Internal Control (IC), vortex thoroughly and centrifuge briefly for 3-5 seconds (s).

(3) Incubate at 55℃ for 30 min.

(4) Add 10 μL 5M NaCl and 10 μL diluted Precipitation solution I, vortex thoroughly and centrifuge briefly for 3-5 s.

4. Extraction of viral nucleic acid

Automated extraction is recommended using rHCDpurify system for viral nucleic acid extraction, and follow the user guide of SHENTEK® Virus DNA & RNA Extraction Kit.

Section I. BVDV/REO-3/PI-3 multiplex RT-qPCR

■ RT-qPCR MIX preparation

1. Determine the number of reaction wells according to the number of test samples and control samples with 2 replicates for each sample generally.

   Number of reaction wells = (1 No Template Control (NTC) + 1 Negative Control Sample (NCS) + 1 Positive Control Sample (PCS) + test samples) × 2

2. Prepare RT-qPCR MIX according to the following table.

Table 2. RT-qPCR MIX preparation

Reagents	Volume/reaction		Volume for 30 reaction (includes 10% overage)
	Test samples	NTC	
5×Multiplex RT-PCR Buffer	4 μL	4 μL	132 μL
Multiplex RT-PCR Enzyme MIX	1 μL	1 μL	33 μL
BV RNA Primer&Probe MIX	3.3 μL	3.3 μL	108.9 μL
Multiplex IPC MIX	1.7 μL	1.7 μL	56.1 μL
VIR Internal Control (IC)*	/	0.5 μL	/
Total volume	10 μL	10.5 μL	330 μL

** If VIR Internal Control (IC) has not been added during sample extraction, 0.5 μL VIR Internal Control (IC) should be added to the RT-qPCR MIX.*

■ RT-qPCR Reaction MIX preparation

1. Incubate all extracted samples at 95°C for 3 min.
2. Vortex each solution thoroughly, then add to each well according to Table 3 and the 96-well plate layout as shown in Table 4.

Table 3. RT-qPCR Reaction MIX preparation in each well

NTC (No Template Control)	10 μL RT-qPCR MIX + 10 μL RNase-Free H_2O
NCS (Negative Control Sample)	10 μL RT-qPCR MIX + 10 μL Extracted NCS
PCS (Positive Control Sample)	10 μL RT-qPCR MIX + 10 μL Extracted PCS
Test Sample	10 μL RT-qPCR MIX + 10 μL of Extracted test sample

Table 4. Example of 96-well plate layout

NCS	NCS				S1	S1				PCS	PCS	A
					S2	S2						B
					S3	S3						C
					S4	S4						D
					S5	S5						E
												F
												G
NTC	NTC											H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example shows 1 NTC, 1 NCS, 1 PCS, and five test samples, with duplicate wells for each sample.*
- *In specific testing, the plate layout can be adjusted based on the sample quantity.*

3. Seal the PCR 8-well strip tubes with caps or the 96-well plate with sealing film. Mix thoroughly using a microplate and microtube shaker, then spin down the reagents for 10 seconds in microcentrifuge and place into the qPCR instrument.

■ RT-qPCR program setting

Taqman probe reporter dyes:

Target	Reporter dye
REO-3	FAM
PI-3	HEX
BVDV	CY5
IC	ROX

Take the SHENTEK-96S Real-time PCR System (software version 8.2.2) as an example, and set the RT-qPCR program as follows:

1. Click on the Experiment Wizard.
2. Select Step 1 on the **Well Plate Edit** page: Select the reaction wells.
3. Select Step 2: Select the **BVDV/REO-3/PI-3 Detection** program in the

project.

4. Click **Start** to run the program on the **Experimental Run** page.

If you have alternative qPCR system (for example ABI 7500), set up the instruments as follows:

1. Run a new method program and select the quantitative PCR assay template.
2. Set the Passive reference fluorescence to 'None'.
3. Run a new Probe template, and type the name “REO-3”. Select FAM in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK. Run a second Probe template, and type the name “PI-3”. Select HEX in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK. Run a third Probe template, and type the name “BVDV”. Select CY5 in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK. Run a fourth Probe template, and type the name “IC”. Select ROX in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK.
4. Set thermal-cycling conditions:
   a. Set the cycling reaction volume to 20 μL.
   b. Set the temperature and the time as follows:

Table 5. RT-qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Reverse transcription	50℃	15:00	1
Activation	95℃	00:30	1
Denaturation	95℃	00:15	40
Annealing/extension	60℃*	01:00	

* Instrument will read the fluorescence signal during this step.

■ Result analysis

Take the SHENTEK-96S Real-time PCR System (software version 8.2.2) as an

example.

1. Select step 3 in the **Edit** page: define the reaction wells, set the sample type for the NTC well as no template control, the PCS well as the positive control, the NCS well as the negative control, and the test sample well as unknown.
2. Click **Analysis** on the **Experimental Analysis** page, and the detection values of NTC, NCS, PCS and test samples can be presented in the **Reaction well Information Table**.

For ABI 7500 Real-Time PCR System instrument (SDS v1.4), please follow the steps below:

1. Select the **Results** tab, then click **Amplification Plot**. In the Analysis Settings window, enter the following settings:
   a. Select **Manual Ct**. In the **Threshold** field, enter 2500.
   b. Select **Manual Baseline**, Start: 3, End: 15.
   c. Click **Analyze**, and check whether it is a specific amplification curve.
2. Select the **Results** tab, and click **Plate** tab, then set tasks for each sample type by clicking on the Task Column drop-down list and then click :
   a. NTC wells: target DNA detector task = NTC.
   b. NCS, PCS, unknown samples wells: target DNA detector task = Unknown.
3. Select **File > > Export > > Results**. In the **Save as type** drop-down list, select **Results Export Files**, then click **Save**.

NOTE: If you use a different instrument or software, please refer to the applicable instrument or software documentation. Usually real-time PCR instrument software automatically exports the data report.

■ Acceptance Criteria

1. Guidance for the control samples as in the following table.

Table 6. Control sample result analysis

Quality control samples	FAM/HEX/CY5	ROX
NTC	Undetected or absence of specific amplification for duplicate runs	Ct<35.00 and specific amplification for duplicate runs
NCS	Undetected or absence of specific amplification for duplicate runs	Ct<35.00 and specific amplification for duplicate runs
PCS	Ct<35.00 and specific amplification for duplicate runs	Ct<35.00 and specific amplification for duplicate runs

The criteria for control sample shall be based on your method validation and considered to satisfy the requirement of LOD (Limit of Detection).

2. Guidance for test samples as in the following table.

Table 7. Test sample result analysis

<table>
<tr><th>FAM
(REO-3)</th><th>HEX
(PI-3)</th><th>CY5
(BVDV)</th><th>ROX
(IC)</th><th>Conclusion</th></tr>
<tr><td>Ct<40.00</td><td>/</td><td>/</td><td rowspan="3">Ct<35.00 and specific amplification for duplicate runs</td><td>Positive for REO-3*</td></tr>
<tr><td>/</td><td>Ct<40.00</td><td>/</td><td>Positive for PI-3*</td></tr>
<tr><td>/</td><td>/</td><td>Ct<40.00</td><td>Positive for BVDV*</td></tr>
<tr><td colspan="3" rowspan="2">Undetected or absence of specific amplification for duplicate runs</td><td>Ct_{sample} - Ct_{NCS} < 2</td><td>Negative for REO-3/PI-3/BVDV</td></tr>
<tr><td>Ct_{sample} - Ct_{NCS} ≥ 2</td><td>Not conclusive, and Presence of PCR inhibitors**</td></tr>
</table>

** Analyzing the detection values of each fluorescent dye channel independently. If any well shows a Ct value <40.00 and shows a specific amplification curve, it can be determined that the viral nucleic acid is presented in the sample.*

*** Compare the ROX Ct value of the sample to the NCS Ct value. If the difference is greater than 2, it indicates inhibition during extraction or detection.*

Note: In an event of specific sample or some abnormalities occur, or results are difficult to determine, please contact us for technical support.

Section II. BPV-3/BAV-3 multiplex qPCR

■ qPCR MIX preparation

1. Determine the number of reaction wells according to the number of test samples and control samples with 2 replicates for each sample generally.

   Number of reaction wells = (1 No Template Control (NTC) + 1 Negative Control Sample (NCS) + 1 Positive Control Sample (PCS) + test samples) × 2

2. Prepare qPCR MIX according to the following table.

Table 8. qPCR MIX preparation

Reagents	Volume/reaction		Volume for 30 reaction (includes 10% overage)
	Test samples	NTC	
Multiplex qPCR Reaction Buffer	15.9 μL	15.9 μL	132 μL
BV DNA Primer&Probe MIX	2.4 μL	2.4 μL	108.9 μL
Multiplex IPC MIX	1.7 μL	1.7 μL	56.1 μL
VIR Internal Control (IC) *	/	0.5 μL	/
Total volume	20 μL	20.5 μL	330 μL

** If VIR Internal Control (IC) has not been added during sample extraction, 0.5 μL VIR Internal Control (IC) should be added to the RT-qPCR MIX.*

■ qPCR Reaction MIX preparation

1. Vortex each solution thoroughly, then add to each well according to Table 9 and the 96-well plate layout as shown in Table 10.

Table 9. qPCR Reaction MIX preparation in each well

NTC (No Template Control)	20 μL qPCR MIX + 10 μL RNase-Free H_2O
NCS (Negative Control Sample)	20 μL qPCR MIX + 10 μL of Extracted NCS
PCS (Positive Control Sample)	20 μL qPCR MIX + 10 μL of Extracted PCS
Test Sample	20 μL qPCR MIX + 10 μL of Extracted test sample

Table 10. Example of 96-well plate layout

NCS	NCS				S1	S1				PCS	PCS	A
					S2	S2						B
					S3	S3						C
					S4	S4						D
					S5	S5						E
												F
												G
NTC	NTC											H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example shows 1 NTC, 1 NCS, 1 PCS, and five test samples, with duplicate wells for each sample.*
- *In specific testing, the plate layout can be adjusted based on the sample quantity.*

2. Seal the PCR 8-well strip tubes with caps or the 96-well plate with sealing film. Mix thoroughly using a microplate and microtube shaker, then spin down the reagents for 10 seconds in microcentrifuge and place into the qPCR instrument.

■ qPCR program setting

Taqman probe reporter dyes:

Target	Reporter dye
BPV-3	FAM
BAV-3	CY5
IC	ROX

Take the SHENTEK-96S Real-time PCR System (software version 8.2.2) as an example, and set the qPCR program as follows:

1. Click on the Experiment Wizard.
2. Select Step 1 on the **Well Plate Edit** page: Select the reaction wells.
3. Select Step 2: Select the **BPV-3/BAV-3 Detection** program in the project.
4. Click **Start** to run the program on the **Experimental Run** page.

If you have alternative qPCR system (for example ABI 7500), set up the instruments as follows:

1. Run a new method program and select the quantitative PCR assay template.
2. Set the Passive reference fluorescence to 'None'.
3. Run a new Probe template, and type the name “BPV-3”. Select FAM in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK. Run a second Probe template, and type the name “BAV-3”. Select CY5 in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK. Run a third Probe template, and type the name “IC”. Select ROX in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK.
4. Set thermal-cycling conditions:

   c. Set the cycling reaction volume to 30 μL.

   d. Set the temperature and the time as follows:

Table 11. RT-qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Activation	95℃	10:00	1
Denaturation	95℃	00:15	40
Annealing/extension	60℃*	01:00	

* Instrument will read the fluorescence signal during this step.

■ Result analysis

Take the SHENTEK-96S Real-time PCR System (software version 8.2.2) as an example.

1. Select step 3 in the **Edit** page: define the reaction wells, set the sample type for the NTC well as no template control, the PCS well as the positive control, the NCS well as the negative control, and the test sample well as unknown.
2. Click **Analysis** on the **Experimental Analysis** page, and the detection values of NTC, NCS, PCS and test samples can be presented in the **Reaction well**

Information Table.

For 7500 Real-Time PCR System instrument (SDS v1.4), please follow the steps below:

1. Select the **Results** tab, then click **Amplification Plot**. In the Analysis Settings window, enter the following settings:
   d. Select **Manual Ct**. In the **Threshold** field, enter 10000.
   e. Select **Manual Baseline**, Start: 3, End: 15.
   f. Click **Analyze**, and check whether it is a specific amplification curve.
2. Select the **Results** tab, and click **Plate** tab, then set tasks for each sample type by clicking on the Task Column drop-down list and then click :
   c. NTC wells: target DNA detector task = NTC.
   d. NCS, PCS, unknown samples wells: target DNA detector task = Unknown.
3. Select **File > > Export > > Results**. In the **Save as type** drop-down list, select **Results Export Files**, then click **Save**.

NOTE: If you use a different instrument or software, please refer to the applicable instrument or software documentation. Usually real-time PCR instrument software automatically exports the data report.

■ Acceptance Criteria

1. Guidance for the control samples as in the following table.

Table 12. Control sample result analysis

Quality control samples	FAM/CY5	ROX
NTC	Undetected or absence of specific amplification for duplicate runs	Ct<35.00 and specific amplification for duplicate runs
NCS	Undetected or absence of specific amplification for duplicate runs	Ct<35.00 and specific amplification for duplicate runs
PCS	Ct<35.00 and specific amplification for duplicate runs	Ct<35.00 and specific amplification for duplicate runs

The criteria for control sample shall be based on your method validation, and

considered to satisfy the requirement of LOD.

2. Guidance for test samples as in the following table.

Table 13. Test sample result analysis

FAM (BPV-3)	**CY5 (BAV-3)**	**ROX (IC)**	**Conclusion**
Ct<40.00	/	Ct<35.00 and specific amplification for duplicate runs	Positive for BPV-3
/	Ct<40.00		Positive for BAV-3
Undetected or absence of specific amplification for duplicate runs		Ct_{sample} - Ct_{NCS} < 2	Negative for BPV-3/BAV-3
		Ct_{sample} - Ct_{NCS} ≥ 2	Not conclusive, and Presence of PCR inhibitors*

** Analyzing the detection values of each fluorescent dye channel independently. If any well shows a Ct value <40.00 and shows a specific amplification curve, it can be determined that the viral nucleic acid is presented in the sample.*

*** Compare the ROX Ct value of the sample to the NCS Ct value. If the difference is greater than 2, it indicates inhibition during extraction or detection.*

Note: In an event of specific sample or some abnormalities occur, or results are difficult to determine, please contact us for technical support.

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Support & Contact

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