SHENTEK

PI-3 Detection Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1506732
Reagents for 50 Reactions

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

1. Product information

■ Product description

The SHENTEK® PI-3 Detection Kit is designed for qualitative detection of Bovine Parainfluenza Virus Type 3 (PI-3, or BPIV-3) contamination in cell banks, virus seed banks, and bovine serum. The kit works together with SHENTEK® Virus DNA & RNA Extraction Kit (Product No.1506730).

The detection assay could reach a sensitivity of 50 copies per reaction, and shows high specificity without cross-amplification with bovine, porcine, and engineering cell lines (CHO, Vero, 293T, MDCK, NS0, and Sf9) or engineering bacteria (*E. coli, Pichia pastoris*) genomes. Combined with SHENTEK® Virus DNA & RNA Extraction Kit, these kits can efficiently recover and detect PI-3 RNA from complex sample matrix 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
PI-3 Primer&Probe MIX	NNC092	200 μL × 1 tube	-20℃, protect from light
RT-qPCR Enzyme MIX	NNC079	100 μL × 1 tube	
IPC MIX	NNC066	150 μL × 1 tube	
5×RT-qPCR Buffer	NNB018	300 μL × 1tube	-20℃
RNase-Free H_2O	NND008	1.2 mL × 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 480 II Real-Time PCR System
- ABI 7500 Real-Time PCR System
- CFX96 Real-time PCR System

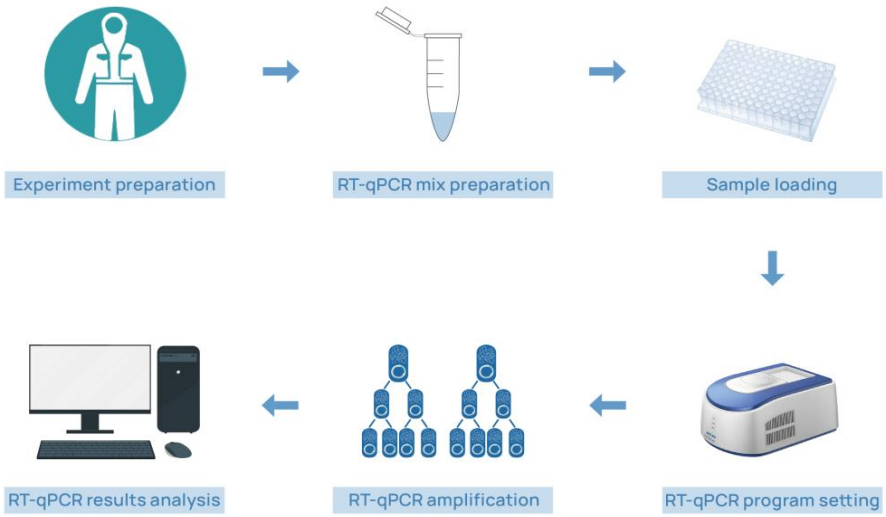
■ Required materials not included in the kit

- Low retention, RNase/DNase-free, sterile microcentrifuge tubes:1.5 mL or 2.0 mL
- PCR 8-well strip tubes with caps or 96-well plates with sealing films
- Low retention, RNase/DNase-free, sterile filter tips: 1000 μL, 200 μL, 100 μL, 10 μL
- SHENTEK® Virus DNA & RNA Extraction Kit (Product No. 1506730)
- SHENTEK® PI-3 Positive Control (Product No. 1506738)
- Pretreatment buffer (NND002), Proteinase K Buffer (NND025), Proteinase K (NND023) and 5M NaCl (NND040), components of the Virus DNA & RNA Extraction Kit (Product No. 1506730)

■ Related equipment

- Benchtop microcentrifuge
- Biosafety cabinet
- 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 biosafety cabinet and follow the BSL-2 laboratory safety guidelines.
3. Use consumables and reagents that are certified nuclease-free.
4. Thaw the kit completely at 2-8°C or melt on ice, vortex and spin briefly.

■ Sample pretreatment

Use the Virus DNA & RNA Extraction Kit for the sample preparation and extraction, with the reagents used being sourced from the extraction kit. Here is an introduction to the sample preparation. For the rest of the detailed steps, please refer to the extraction user guide (Product No. 1506730).

1. Preparation of control samples

Positive Control Samples (PCS)

Take a PI-3 Positive Control and follow the same extraction procedure used for the test samples.

Negative Control Samples (NCS)

Take an equal volume of RNase-Free H_2O or 1×PBS, and follow the same

extraction procedure used for the test samples.

2. Preparation of test samples

For cell culture and bovine serum samples

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

(2) Add Pretreatment buffer (NND002) to the sample at the 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℃, and carefully transfer the supernatant to a new microcentrifuge tube.

NOTE: Other types of samples can be directly processed for sample digestion. For special type matrix samples, please consult our technical support!

3. Samples digestion

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

(2) Incubate the samples at 55℃ for 30 min.

(3) Add 10 μL 5M NaCl (NND040) and 10 μL diluted Precipitation solution I (Please see Before each use of the kit - Precipitation solution I dilution of Virus DNA & RNA Extraction Kit User Guide).

(4) Vortex thoroughly and centrifuge briefly for 3-5 seconds.

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.

■ 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)**
PI-3 Primer&Probe MIX	3.7 μL	122.1 μL
5×RT-qPCR Buffer	4 μL	132 μL
RT-qPCR Enzyme MIX	1 μL	33 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	10 μL	330 μL

■ RT-qPCR Reaction MIX preparation

1. Vortex each solution to mix well. Then add the solution to each well according to Table 3, and the plate layout of 96-well is 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 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.*

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

■ RT-qPCR program setting

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 **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, set up the instruments as follows:

1. Run a new method program and select the quantitative PCR assay template.
2. Run a new Probe template, and type the name **PI-3.** Select **FAM** in the Reporter Dye drop-down list and select **none** in the Quencher Dye drop-down list, then click **OK**. Select **VIC** in the Reporter Dye drop-down list and select **none** in the Quencher Dye drop-down list, then click **OK**. Select the detection reference fluorescence as ROX (optional).
3. 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	45
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** on the Well Plate 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:
   a. Select **Manual Ct**.
   b. In the Threshold field, enter 0.02.
   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, refer to the applicable instrument or software documentation. Usually real-time PCR instrument software automatically export 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	VIC
NTC	Ct≥40.00 or absence of specific amplification for duplicate runs	Ct<35.00 and specific amplification for duplicate runs
NCS	Ct≥40.00 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 method validation data, 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

FAM	VIC	Conclusion
Ct<40.00 (at least one well) and specific amplification	Ct<40.00 and specific amplification for duplicate runs	Positive
	Ct≥40.00 or absence of specific amplification for duplicate runs	Presumptive positive and presence of PCR inhibitors
Ct≥40.00 or absence of specific amplification for duplicate runs	Ct<40.00 and specific amplification for duplicate runs	Negative
	Ct≥40.00 or absence of specific amplification for duplicate runs	Not conclusive, and presence of PCR inhibitors*

** If the VIC signal is inhibited, it is necessary to repeat the sample preparation or appropriately remove the inhibitors during the sample preparation and repeat the assay.*

NOTE: In an event that the sample is special, or some abnormalities occur, and results difficult to determine, please contact us for technical support.

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Support & Contact

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