SHENTEK

Vesivirus 2117 Detection Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1506743
Reagents for 50 Reactions

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

1. Product information

■ Product description

The SHENTEK® Vesivirus 2117 Detection Kit is designed for qualitative detection of Vesivirus 2117 contamination in mammalian cell lines such as CHO cells used in biopharmaceutical production processes. 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 engineered cell lines (CHO, VERO, 293T, MDCK, NS0, and Sf9) or engineered bacteria (*E. coli, Pichia pastoris*) genomes. Vesivirus 2117 Positive Control and VIR Internal Control (IC) are provided to ensure reliable results. Combined with SHENTEK® Virus DNA & RNA Extraction Kit, these kits can efficiently recover and detect Vesivirus 2117 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
VIR Internal Control (IC)	NNA057	600 µL × 1 tube	-20℃
5×RT-qPCR Buffer	NNB018	300 µL × 1 tube	
RNase-Free H_2O	NND008	1.2 mL × 1 tube	
RT-qPCR Enzyme MIX	NNC079	100 µL × 1 tube	-20℃, protect from light
VV-2117 Primer&Probe MIX	NNC123	170 µL ×1 tube	
VIR IPC MIX	NNC107	100 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

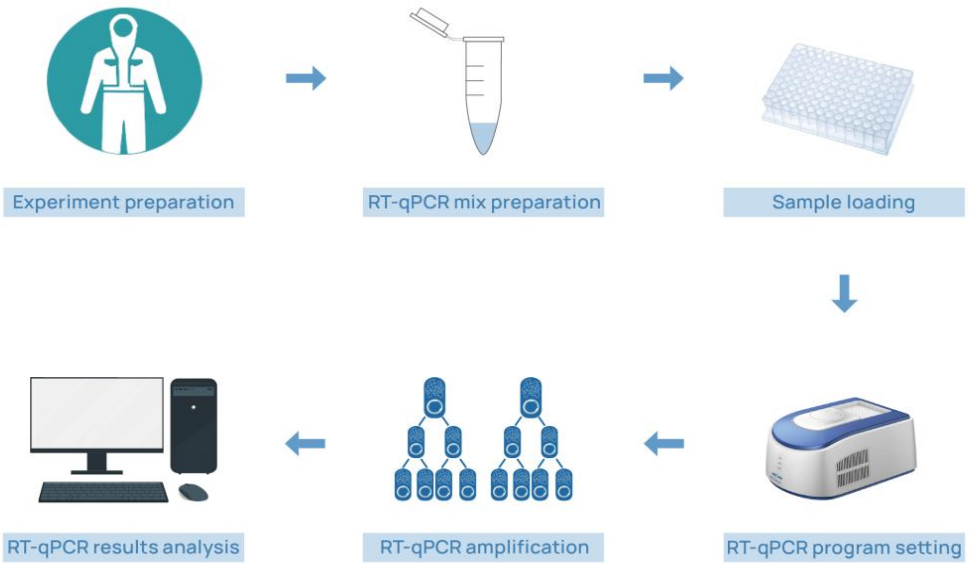
■ Required materials not included in the kit

- Nonstick, RNase/DNase-free, sterile microcentrifuge tubes, 1.5 mL and 2.0 mL
- PCR 8-well strip tubes with caps or 96-well plates with sealing films
- Low retention, sterile filter tips: 1000 µL, 200 µL, 100 µL and 10 µL
- SHENTEK® Virus DNA & RNA Extraction Kit (Product No. 1506730)
- SHENTEK® Vesivirus 2117 Positive Control (Product No. 1506743-R01)

■ 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 nuclease-free.
4. Thaw the kit completely at 2-8°C or melt on ice, vortex and spin briefly.

■ Sample pretreatment

Use Virus DNA & RNA Extraction Kit for Vesivirus 2117 RNA extraction.

1. Preparation of test samples

For cell culture and bovine serum samples

(1) Collect 100-200 μL of the cell culture ($\leq 10^7$ cell) or serum 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℃, carefully transfer the supernatant to a new microcentrifuge tube.

2. Preparation of control samples

Positive control samples (PCS)

Take a Vesivirus 2117 Positive Control and follow the same extraction procedure as 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 as for the test samples.

3. Samples digestion

(1) Add 100 μL proteinase K buffer and 50 μL of proteinase K.

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

(3) Incubate at 55℃ for 30 min.

(4) Add 10 μL 5M NaCl and 10 μL of diluted Precipitation solution I, vortex 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.

■ 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**	**e.g. Test samples**
5×RT-qPCR Buffer	4 μL	4 μL	132 μL
RT-qPCR Enzyme MIX	1 μL	1 μL	33 μL
VV-2117 Primer&Probe MIX	3.3 μL	3.3 μL	108.9 μL
VIR 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. Vortex each solution and 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 of purified NCS
PCS (Positive Control Sample)	10 μL RT-qPCR MIX + 10 μL of purified PCS
Test Sample	10 μL RT-qPCR MIX + 10 μL of purified 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 "VV-2117 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 “VV-2117”. 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 following:

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 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 Settingswindow, 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	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 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<35.00 and specific amplification for duplicate runs	Positive
Undetected or absence of specific amplification for duplicate runs	Ct_{sample} - Ct_{NCS} < 2	Negative
	Ct_{sample} - Ct_{NCS} ≥ 2	Not conclusive, and Presence of PCR inhibitors*

** Compare the VIC Ct value of the sample to the NCS Ct value. If the difference is greater than 2, it indicates inhibition in extraction or detection. Retest or remove the potential inhibitors appropriately to confirm the virus contamination.*

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

■ References

- FDA. Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications 2010
- 9 CFR 113.47 Detection of extraneous viruses by the fluorescent antibody technique
- USP <1050> Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
- EP <5.2.3> Cell substrates for the production of vaccines for human use
- EP <2.6.16> Tests for extraneous agents in viral vaccines for human use
- EP <5.1.7> Viral safety
- ChP <3302> Exogenous viral factor screening method
- JP <G3-13-141> Basic Requirements for Viral Safety of Biotechnological/ Biological Products listed in Japanese Pharmacopoeia
- FDA. Q5A(R2) Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
- EMA GUIDELINE ON VIRUS SAFETY EVALUATION OF BIOTECHN OLOGICAL INVESTIGATIONAL MEDICINAL PRODUCTS

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Support & Contact

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