SHENTEK

MVM Detection Kit

User Guide

Version: A/2
For Research Use Only
Product No.: 1506742
Reagents for 50 Reactions

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

1. Product information

■ Product description

The SHENTEK® MVM Detection Kit is designed for qualitative detection of Minute Virus of Mice (covering i, p, m and c strains) contamination in mouse-derived cell lines used in biopharmaceutical production processes, for example CHO, BHK, and NS0 cells. The kit works together with SHENTEK® Virus DNA & RNA Extraction Kit (Product No.1506730).

The detection assay is capable of detecting four MVM strains (i, p, m and c), and could reach a sensitivity of 50 copies per reaction, meanwhile shows high specificity without cross-amplification with engineered cell lines (CHO, VERO, 293T, MDCK, NS0, and Sf9) or engineered bacteria genomes (*E. coli, Pichia pastoris*). MVM Positive Control and VIR Internal Control (IC) are provided to ensure reliable results. Combined with SHENTEK® Virus DNA & RNA Extraction Kit, this kit can efficiently recover and detect MVM DNA from complex sample matrices, such as cells and serum.

The kit is for Research Use Only and is not for use in diagnostic procedures or for therapeutic purposes in humans or animals.

■ Kit components and storage

WARNING: Read the Material Safety Data Sheets (MSDS) 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 DNA Internal Control (IC)	NNA058	600 µL × 1 tube	-20℃
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 1 tube	
qPCR Master MIX	NNB023	850 µL × 1 tube	-20℃, protect from light
MVM Primer&Probe MIX	NNC106	150 µL × 1 tube	
VIR IPC MIX	NNC107	100 µL × 1 tube	

Note: According to USP <659>, items with recommended storage temperatures not exceeding –20°C shall be stored within ± 10°C (–30°C to –10°C). Short-term storage below –30°C is acceptable when supported by stability data.

The kit components can be stored under the 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

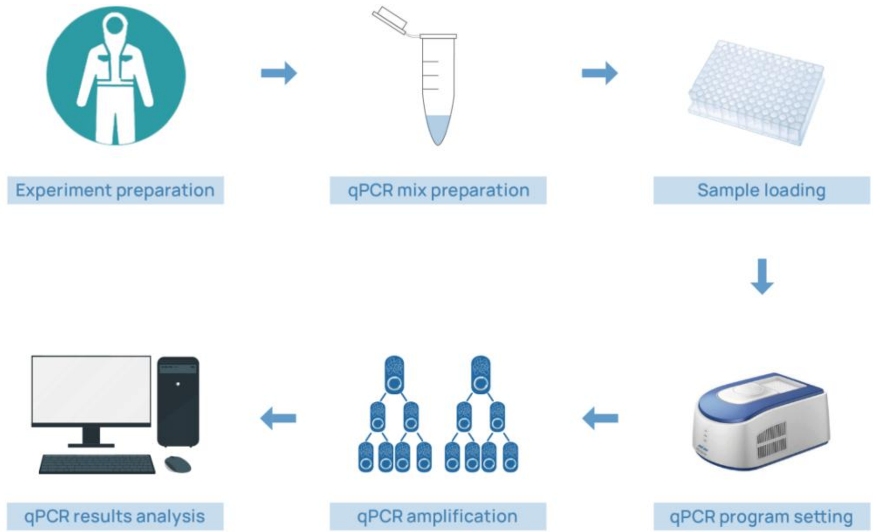
■ Required materials not included in the kit

- Low retention, DNase-free, sterile microcentrifuge tubes, 1.5 mL
- 96-well qPCR plates with sealing film or 8-tube PCR strips with caps
- Low retention, RNase/DNase-free, sterile tips: 1000 µL, 200 µL, 100 µL and 10 µL
- SHENTEK® Virus DNA & RNA Extraction Kit (Product No. 1506730)
- SHENTEK® MVM Positive Control (Product No. 1506747)
- Pretreatment buffer (NND002), Proteinase K Buffer (NND025), Proteinase K (NND023), Precipitation solution I (NND003) and 5M NaCl (NND040), components of the Virus DNA & RNA Extraction Kit (Product No. 1506730)

■ Related equipments

- Benchtop microcentrifuge
- Microplate and microtube shaker
- Vortex mixer
- rHCDpurify System
- 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 and centrifuge briefly.

■ Sample pretreatment

Use Virus DNA & RNA Extraction Kit for MVM DNA extraction.

1. Preparation for test samples

For cell matrix and bovine serum samples

(1) Collect 200 μL of the cell culture (≤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°C, 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!*

2. **Preparation of control samples**

Positive control samples (PCS)

Take 1 tube of Positive Control (100 μL) and follow the same extraction procedure as for the test samples.

Negative control samples (NCS)

Take an equal volume of DDB or 1×PBS as the test sample, and follow the same extraction procedure used for the 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 DNA Internal Control (IC), vortex thoroughly and briefly centrifuge for 3-5 seconds (s).

(3) Incubate the samples at 55°C for 30 min.

(4) Add 10 μL 5M NaCl (NND040) and 10 μL diluted Precipitation solution I (NND003). (Please check the following user guide before each use of the kit: Precipitation solution I dilution of Virus DNA & RNA Extraction Kit User Guide).

(5) 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.

■ 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 2. qPCR MIX preparation

Reagents	**Volume/reaction**		**Volume for 36 reactions (includes 10% overage)**
	Test samples	**NTC**	
qPCR Master MIX	15.9 µL	15.9 µL	636 µL
MVM Primer&Probe MIX	2.4 µL	2.4 µL	96 µL
VIR IPC MIX	1.7 µL	1.7 µL	68 µL
VIR DNA Internal Control (IC) *	/	0.5 µL	/
Total volume	20 µL	20.5 µL	800 µL

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

■ qPCR Reaction MIX preparation

1. Vortex each solution and mix well. Then add the solution to each well according to Table 3, and refer to 96-well plates layouts shown in Table 4.

Table 3. qPCR Reaction MIX preparation in each well

NTC (No Template Control)	20 µL qPCR MIX + 10 µL DDB
NCS (Negative Control Sample)	20 µL qPCR MIX + 10 µL of purified NCS
PCS (Positive Control Sample)	20 µL qPCR MIX + 10 µL of purified PCS
Test Sample	20 µL qPCR MIX + 10 µL of purified test sample

Table 4. Example of 96-well plate layouts

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	

Note: This example shows 1 NTC, 1 NCS, 1 PCS, and five test samples, with duplicate wells for each sample.

Note: In specific testing, the plate layout can be adjusted based on the sample quantity.

2. Seal the 8-tube PCR strips with caps or 96-well qPCR plates with sealing film. Mix the contents of the wells in microplate shaker, then spin down the plate for 10 seconds in microcentrifuge and place it in the qPCR instrument.

■ qPCR program setting

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 "**MVM 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 "MVM". 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 30 μL.
   b. Set the temperature and time as follows:

Table 5. qPCR cycling 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 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**.
   b. In the **Threshold** field, enter value 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. Normally, real-time PCR instrument software will export the data reports automatically.

■ 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 your method validation, and considered to satisfy the requirement of DL (Detection Limit).

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} - $Ct_{NCS} \geq 2$	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.

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Support & Contact

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