SAFENSURE™ Minute Virus of Mice（MVM）Detection Kit (qPCR)

User Manual V1.0

Catalog Number: OPA-S104

Assay Tests: 100 Tests

IMPORTANT: Please carefully read this user guide before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures.

Contents

US and Canada: **Tel**: +1 800-810-0816

Asia and Pacific: **Tel**: +86 400-682-2521

Web: http://www.acrobiosystems.com

E-mail: order@acrobiosystems.com

Product Information

This kit is intended for the qualitative detection of Minute Virus of Mice (MVM) DNA. It is applicable to, but not limited to, detecting MVM contamination in the following scenarios: recombinant proteins/monoclonal antibodies/fusion proteins, vaccines, recombinant enzymes, cell banks (primarily host cells such as CHO, NS0, and BHK-21), as well as raw and auxiliary materials (specifically murine-derived cells, murine-derived auxiliary materials, and mouse-derived preparations), plus intermediates and final products of other biological products.

The kit is based on TaqMan qPCR technology and specifically detects MVM strains i, p, m, and c. It avoids interference from non-target microorganisms, common engineered cell lines, and cell culture matrix components. UNG enzyme has been incorporated into the qPCR master mix to effectively prevent false positives caused by non-specific amplification products. An internal control (MVM Internal Control) is also provided, which amplifies stably in the qPCR reaction without cross-interference with the target. It can be added during sample extraction to monitor extraction efficiency, or during qPCR setup to monitor for amplification inhibition.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Important Notes

1. This kit is for research use only and is not intended for clinical diagnosis.
2. The kit should be stored under the conditions specified in the manual and used before its expiration date.
3. All components of the kit must be completely thawed at room temperature or 2–8°C before use.
4. Please strictly follow the instructions. All components must be used with the reagents provided in this kit to ensure optimal detection results.
5. Laboratory compartmentalization: It is recommended that PCR master mix preparation, sample extraction and DNA addition, and PCR amplification be performed in separate areas. Ensure the cleanliness of each experimental zone to minimize cross-contamination.
6. To prevent exogenous contamination, strictly control the environment, personnel, and operational practices: Operators must wear powder-free gloves and masks. Consumables requirements: Use disposable, sterile, nuclease-free, low-adhesion consumables with filter tips. Operational practices: Change tips promptly when pipetting to avoid cross-contamination; avoid leaving tubes open for extended periods. Clean the workbench before and after operation, and use a nucleic acid decontaminant if necessary.
7. Our company is committed to ensuring the quality of each kit. Please note that our warranty does not cover sample loss that may occur during experimental operations. It is recommended that users assess their experimental needs in advance and reserve sufficient backup samples.

US and Canada: **Tel**: +1 800-810-0816
Asia and Pacific: **Tel**: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Components and Storage

Table 1. Kit Components

Contents	Colors	Amount	Storage
MVM qPCR Master Mix		750 µL×2	-15 ~ -30℃ Note: Protect the MVM Assay Mix & MVM qPCR Master Mix from light.
MVM Assay Mix		400 µL×1	
MVM Positive Control		1 mL×1	
MVM Internal Control		1 mL×1	
DNase/RNase-Free Water		1.5 mL×3	

The kit should be stored at -15°C to -30°C and is valid for 18 months from the date of manufacture (see outer package label for details).

US and Canada: **Tel**: +1 800-810-0816
Asia and Pacific: **Tel**: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Required materials not supplied

Table 2. Required materials

Category	Item	Experimental Recommendation
Equipment	Real-time PCR instrumentation	FAM Channel
	Automated Nucleic Acid Extraction System	(Optional) Acro OPE-32S
	Vortex	2500~3000 rpm
	Mini centrifuge	3000~6000 rpm
	96-well plate centrifuge	3000~6000 rpm
	Pipettes	1000 µL, 100 µL, 20 µL, and 10 µL
Consumables	qPCR 8-tube strips with caps, or 96-well plates with sealing films	For qPCR Instruments, Nuclease-Free, DNA-Free
	Pipette tips	Nuclease-Free, DNA-Free, with Filter
	Low-binding centrifuge tubes	Nuclease-Free, DNA-Free

Compatible Real-Time PCR System (including but not limited to):

- ABI 7500/7500 Fast Real-Time PCR System
- ABI QuantStudio® 5 Real-Time PCR System
- Roche Light Cycler 480 II
- Bio-Rad CFX96 Real-Time PCR System
- SLAN-96S Real-Time PCR System

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Abbreviation Table

Table 3. Abbreviation Table

Abbreviation	Full Name
MVM	Minute Virus of Mice
CHO	Chinese Hamster Ovary
Ct	Cycle Threshold
NTC	No Template Control
PAC	Positive Amplification Control
NEC	Negative Extraction Control
PEC	Positive Extraction Control
Und.	Undetermined

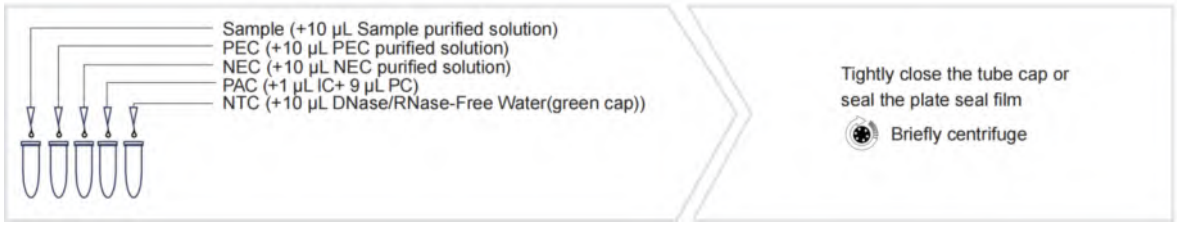
US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Workflow

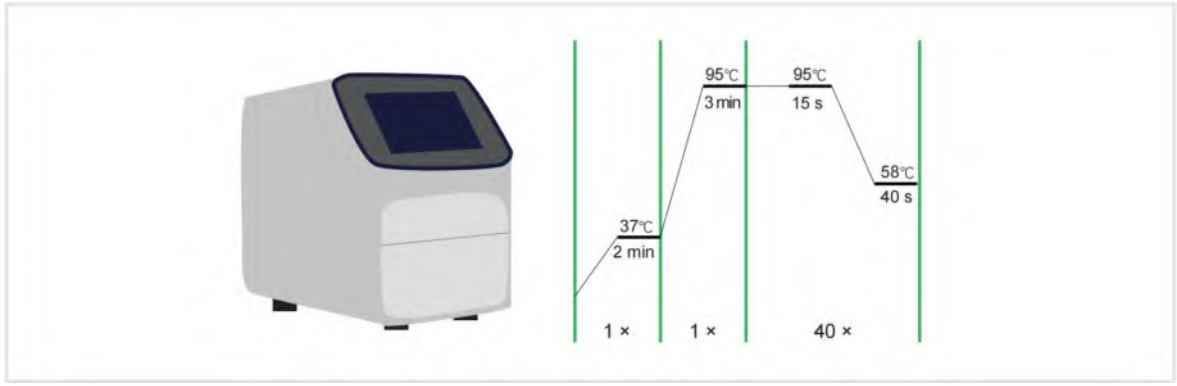
Step 1 Prepare the Reagents

Step 2 Prepare the qPCR Working Mix

Step 3 Add Samples and Controls



Step 4 Set up and Run qPCR Program



+ add vortex centrifuge

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Protocol

1. Pre-Experiment Preparation

Take the reagents out of the refrigerator and place them on ice to thaw. Vortex mix for 20-30 seconds and then briefly centrifuge.

2. Experimental Control Design

To ensure accurate interpretation of test results, the following controls should be included in the experiment.

Table 4. Experimental Control Designs

Controls	Samples	Purpose	Number of PCR Replicates
No Template Control (NTC)	DNase/RNase-Free Water	Monitor the contamination of qPCR reaction system preparation and amplification processes.	3
Positive Amplification Control (PAC)	MVM Positive Control	Monitor the amplification efficiency.	3
Negative Extraction Control (NEC)	Extraction of sample diluent or corresponding cellular matrix for test sample	Monitor the contamination of extraction processes (including reagents, equipment, work areas and procedure).	3
Positive Extraction Control (PEC)	Extraction of MVM Positive Control	Monitor the efficiency of extraction and amplification.	3

3. Prepare the qPCR Working Mix

3.1 Calculation of Reactions:

Determine the number of reactions required based on the total samples and controls to be tested. Three replicate reactions per sample are recommended.

3.2 Calculation of qPCR Working Mix Reactions (**N**):

qPCR Working Mix reactions (**N**) = (number of reactions + 2 or 3), where 2 or 3 extra reactions are included as a compensation for pipetting loss.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

3.3 Mix the reagents gently by brief vortexing and centrifuge briefly before use. Prepare the qPCR Working Mix according to Table 5, mix it thoroughly, and centrifuge briefly.

Table 5. qPCR Working Mix Preparation

Kit Reagents	Volume for 1 reaction	Volume for Working Mix
MVM qPCR Master Mix	15 µL	15 µL×**N**
MVM Assay Mix	4 µL	4 µL×**N**
DNase/RNase-Free Water	1 µL	1 µL×**N**

NOTE: *If the MVM Internal Control (IC) was not added during extraction, replace the 1 µL of DNase/RNase Free Water with 1 µL of MVM Internal Control (IC) in the qPCR Working Mix.*

3.4 qPCR Reaction System Preparation: Pipette 20 µL of qPCR Working Mix into each well according to the plate layout, and add the corresponding sample to each well.

Table 6. qPCR Reaction System

Reaction Well	qPCR Working Mix	Template	Total Volume
NTC	20 µL	10 µL DNase/RNase-Free Water	30 µL
PAC	20 µL	1 µL MVM Internal Control+ 9 µL MVM Positive Control	30 µL
NEC	20 µL	10 µL NEC purified solution	30 µL
PEC	20 µL	10 µL PEC purified solution	30 µL
Test Sample	20 µL	10 µL Sample purified solution	30 µL

NOTE: *For the positive amplification control (PAC), MVM Internal Control must be added separately when adding the template. For all other extracted samples, it is recommended to add MVM Internal Control during extraction.*

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

4. qPCR Setup and Operation

Using the ABI 7500 system with 7500 software v2.4 as an example:

4.1 Click 'New experiment', enter a name for the experiment and select '**Quantitation Standard Curve**', '**TaqMan** reagents' and '**Standard**' mode.

4.2 In the Plate Setup, create the targets according to the following steps:

a. Enter the Target Name "**MVM**", select FAM in the Reporter Dye drop-down list. Select (None) in the Quencher Dye drop-down list.

b. Enter the Target Name "**MVM Internal Control**", select CY5 in the Reporter Dye drop-down list. Select (None) in the Quencher Dye drop-down list.

c. Select **ROX** in the Passive Reference Dye drop-down list.。

4.3 Set up the test samples and controls as shown in the Plate Layout.

4.4 Target Setup: Arrange the plate layout based on the experimental requirements. It is recommended to place the NTC, NEC, PEC, PAC, and test samples in separate zones during the design and layout of the reaction wells to prevent cross-contamination and ensure accurate test results.

Table 7. Plate Layout Example

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC										PAC	
B	NTC										PAC	
C	NTC				S1	S1	S1				PAC	
D					S2	S2	S2					
E	NEC				S3	S3	S3				PEC	
F	NEC										PEC	
G	NEC										PEC	
H												

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

4.5 Set up the qPCR reaction program according to the following table.

Table 8. qPCR Reaction Program

Step	Cycles	Temperature	Time	Signal Collection
1	1×	37℃	2 min	No
2	1×	95℃	3 min	No
3	40×	95℃	15 s	No
		58℃	40 s	**Yes**

4.6 Set the reaction volume to **30 µL**, click "Start Run" in the Run interface to start the qPCR run, and analyze the results after completion.

US and Canada: **Tel**: +1 800-810-0816
Asia and Pacific: **Tel**: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Analyze the results

1. Analyze Settings

1.1 After the qPCR run is finished, use the general procedure to analyze the results. For ABI 7500, set up the threshold value for FAM at 0.2 and set auto baseline; the threshold value for CY5 is 0.05 and select auto baseline.

1.2 For other instruments, the setting of parameters should be adjusted according to the specific instrument user guide and software version.

2. Results Judgement Criteria

Table 9. Control Sample Results Judgement Criteria

Control Samples	FAM	CY5	Result
NTC	Und. or Ct Value > 39	Und. or Ct Value > 39	QC Acceptable
PAC	Ct Value≤39	Ct Value≤39	QC Acceptable
NEC	Und. or Ct Value > 39	Ct Value≤39	QC Acceptable
PEC	Ct Value≤39	Ct Value≤39	QC Acceptable

Table 10. Test Sample Results Judgement Criteria

Test Samples	FAM	CY5	Result
Tests Samples	Ct Value≤39	Ct Value≤39	**Positive**
		Und. or Ct Value > 39	Inhibition (extraction/amplification) → retest.
	Und. or Ct Value > 39	Ct Value≤39	**Negative**
		Und. or Ct Value > 39	Inhibition (extraction/amplification) → retest.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Appendix

Appendix A: FAQ

Common Issues	Causes and Solutions
During analysis, the CY5 channel of the extracted sample was not detected	**Possible cause**: Forgot to add MVM Internal Control during sample extraction. **Recommendation**: When extracting each sample, add MVM Internal Control to monitor the extraction process.
What is the Ct value range for amplification of the MVM Internal Control?	After verification in our laboratory, the Ct value range for the MVM Internal Control is typically 28–33. Variations may exist among different laboratories. The Ct value of the MVM Internal Control may differ slightly due to factors such as differences in extraction procedures and pipetting errors. As long as the result meets the acceptance criterion——MVM Internal Control (CY5 channel) Ct value ⩽ 39——it is considered acceptable.

Appendix B: Recommended Consumables

Recommended Consumables	Cat. No.	Brand
2.0 mL Low-binding centrifuge tubes	72.695.700	SARSTEDT
1.5 mL Low-binding centrifuge tubes	72.706.700	SARSTEDT
Nuclease-Free Water	OPA-R026	ACRO

US and Canada: **Tel**: +1 800-810-0816
Asia and Pacific: **Tel**: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com