Lymphocytic Choriomeningitis Virus (LCMV) REAL-TIME PCR KIT INSTRUCTION

INTRODUCTION

Lymphocytic Choriomeningitis Virus (LCMV), a member of the family *Arenaviridae*, is a spherical enveloped virus with a diameter between 60 and 300 nm. The helical nucleocapsid contains an RNA genome consisting of two negative single-stranded RNA segments. LCMV infection in mice can result in either a persistent, subclinical infection or an acute infection with severe symptoms. Persistent infections, often acquired in utero or shortly after birth, lead to lifelong virus shedding and immune complex disease (glomerulonephritis). Acute infections, occurring later in life, can cause meningoencephalitis (inflammation of the brain and meninges). The virus is resistant to drying and therefore humans can become infected by inhaling infectious aerosolized particles of rodent urine, feces, or saliva, by ingesting food contaminated with virus, by contamination of mucous membranes with infected body fluids, or by directly exposing cuts or other open wounds to virus-infected blood.

PRINCIPLE OF THE TEST

This VRL kit is designed for specific *in vitro* detection of the LCMV genome. It employs a reverse transcriptase function to convert the RNA-based genome to cDNA, as well as a Taq polymerase for cDNA amplification. The reaction contains a hydrolysis (or TaqMan®) probe that supports quantitative analysis via FAM fluorescence.

The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with over 95% of the NCBI database reference sequences available at the time the test was designed.

HANDLING

- Each kit contains enough reagents to perform 200 tests.
- After receiving, please store different components of the kit at the proper temperatures.
- All reagents can be used before the expiration date printed on the box.
- PCR reagents should be aliquoted when necessary to avoid more than 2 freezing and thawing cycles.

NOTE: FOR RESEARCH USE ONLY

MATERIALS PROVIDED

Kit Components	Amount	Storage Temp.
LCMV primer/probe mix*	4 vials	Dry: 4 ℃
		After reconstitution: -20 ℃
LCMV 10X positive control	2 vials	Dry: 4 ℃
		After reconstitution: -20 ℃
RNase/DNase free water	1 mL	-20 ℃ to Ambient
2X RT-qPCR Reaction Mix	2 x 1 mL	-20 ℃
TE Buffer	1 mL	-20 ℃ to Ambient

*: Light sensitive: Protect from light

REQUIRED BUT NOT PROVIDED

1. Extraction Kits (see details below)
2. Real-time PCR instrument.
3. Microcentrifuge
4. Pipettes (0.5 µL - 1 mL) with sterile filter tips
5. 1.5 - 2 ml sterile micro centrifuge tubes
6. 96-well, thin wall PCR plates
7. Individual PCR tubes, 8-tube strip
8. Powder-free gloves

WARNINGS AND PRECAUTIONS

- This test must be carried out by skilled personnel.
- Strictly divide sample and reagent preparation areas physically from the amplification room/area.
- Samples should be regarded as potentially infectious materials.
- Store positive and/or potentially positive materials separated from all other components of the kit.
- Do not use the kit after its expiration date.
- Always use sterile filter tips for pipetting.
- Pipettes, vials and other working materials should not circulate among separate workspaces.
- Wear separate coats and gloves in each area.
- Routinely decontaminate your pipettes and benches with disinfectants such as bleach, DNA AWAY™, or RNase AWAY™.

➢ RNA extraction

1. The following kits are recommended for preparation of extracted RNA for this test. However, any process that yields high quality RNA with minimal PCR inhibitors is acceptable:

   Kit for RNA extraction from tissue: Millipore Sigma, High Pure RNA tissue kit, Part No. 12033674001(50 extractions/kit).

   Kit for RNA extraction from serum: Qiagen, QIAamp Viral RNA mini kit, Part. No. 52906(50 extractions/kit).

2. **It is recommended to store extracted DNA/RNA at -20℃.**

➢ LCMV Real-time PCR

■ Reagent Resuspension

1. To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Filtered tips are recommended for all pipetting steps.
2. Pulse-spin each tube in a microcentrifuge before opening.
3. Reconstitute the kit components in the RNase/DANase-free water supplied, according to the following table:

Component	Volume water
LCMV primer/probe mix*	40 µL (each vial)
10X LCMV positive control	50 µL (each vial)

*: Light sensitive, please protect from light

4. To ensure complete resuspension, vortex each tube thoroughly.
5. Resuspend the primer/probe mix vials and LCMV positive control vials sequentially, only as needed.
6. Note: The positive control contains high-copy number template and is a significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the pre-PCR components (i.e., primer/probe mix).

■ qPCR setup protocol

1. For each extracted sample, prepare a reaction mixture according to the table below. Include sufficient reactions for positive and negative controls:

   Per reaction

Component	Volume
LCMV primer/probe mix	0.8 µL
2X RT-qPCR Reaction Mix	10 µL
RNase/DNase Free water	4.2 µL
FINAL VOLUME	**15 µL**

2. Pipette 15 µL of reaction mix into each well/plate

according to your qPCR experimental plate setup.

3. Pipette 5 µL of RNA-extracted sample into each well, according to your experimental plate setup.
4. Preparation of 1X LCMV positive control: Dilute 0.8 µL 10X LCMV positive control with 7.2 µL TE buffer. Load 5 µL into the appropriate well.
5. For blank control wells, use 5 µL RNase/DNase free water.
6. The final volume in each well must be 20 µL.
7. If a standard curve is to be included for quantitative analysis, prepare a standard curve dilution series:
   a. Pipette 18 µL into 4 tubes and label tubes 1-4.
   b. Pipette 2 µL 10X LCMV positive control into tube 1.
   c. Vortex thoroughly, then briefly spin down the contents.
   d. Change pipette tip and pipette 2 µL from tube 1 into tube 2.
   e. Vortex thoroughly, then briefly spin down the contents.
   f. Repeat steps d. and e. to complete the dilution series

Standard Curve	Copy Number
10X LCMV Positive control	$1x10^4$ per reaction
Tube 1	$1x10^3$ per reaction
Tube 2	$1x10^2$ per reaction
Tube 3	$1x10^1$ per reaction
Tube 4	$1x10^0$ per reaction

   g. Pipette 5 µL of standard template into each well for the standard curve according to your experimental plan setup.

■ qPCR setup suggestions

8. Prepare and aliquot primer/probe mix inside a biosafety cabinet (BSC) or PCR workstation in a pre-PCR room/area. That is, separate from where samples, PCR positive controls are handled, and separate from the amplification room. Keep all reagents cool (or on ice) at each experimental step.
9. In the pre-PCR room/area, prepare and mix the reaction mixture thoroughly, then pipette 15 µL into each reaction tube or well of a 96-well plate.
10. Transfer the reaction tubes or plate into the PCR assembly or amplification room. In a BSC or PCR workstation add the blank control, extracted RNA sample, and positive control to the reaction mix. Use tips with aerosol filters to add sample and positive controls
11. Add 5 µL in the following order: Blank control ultrapure water, sample RNA, and positive control(s) into the designated tube/well, mix carefully by pipetting.
12. Centrifuge tubes briefly to spin down the contents.

■ qPCR run protocol

	Step	Temp	Time	Cycles
1	Reverse Transcription	50 °C	10 min	1
2	Initial Denaturation	95 °C	3 min	1
3	Denaturation	95 °C	15 sec	45
	Annealing/Extension	60 °C	3 min	

Fluorogenic data should be collected through the FAM channel

1. For basic information regarding the setup and programming of your real-time PCR instrument, please refer to the user manual.
2. Load the tubes/plate in the thermal cycler and start the run.

INTERPRETATION OF RESULTS

The results are valid when the controls and the samples meet all the requirements as follows:

✧ Expected results for controls

Controls	FAM channel	
	Cq Value	Amplification curve
Blank Control (BC)	>38.0	-
1X Positive Control (PC) ($1x10^3$ copies per reaction)	22 - 30	Typical (S-shaped curve)

Note: If Cq of blank control is ≤38.0, then the test is invalid and must be repeated.

✧ Results for samples

FAM Channel		Results
Cq Value	Amplification curve	
≤38.0	Typical	Positive
None detected	-	Negative
Or >38.0		