Lymphocystis Disease Virus (LCDV) LAMP Detection Kit (Fluorometric)

Catalog No	Size
• DL03B100	100 Reaction
• DL03B1000	1000 Reaction

INSTRUCTIONS FOR USE

1. PRODUCT NAME:
Lymphocystis Disease Virus (LCDV) LAMP Detection Kit (Fluorometric)

2. MANUFACTURER:
SuGenomik Biyoteknoloji, METU Technopolis, Yenimahalle, Ankara.

3. INTENDED USE
Lymphocystis Disease Virus (LCDV) LAMP Detection Kit (Fluorometric) is a qualitative in vitro diagnostic test for the detection of nucleic acids from LCDV in fish samples. This product has been designed to target *MCP* gene of LCDV.

4. KIT CONTENT

Content	100 Reaction	1000 Reaction
LCDV LAMP Master Mix	1750 µl	1750 µl X10
LCDV LAMP Primer Mix	250 µl	250 µl X 10
LCDV LAMP Positive Control	50 µl	500 µl
LCDV LAMP Negative Control	50 µl	500 µl

5. STORAGE CONDITIONS
Store at -20±5°C away from light for 12 months.

6. SPECIMEN PREPARATION AND STORAGE
- It is recommended that specimens shall be used immediately after collection. However, the specimens can be stored maximum 4 days at 2-8℃ in a fridge or maximum 1 year at −20℃ in a freezer if immediate use is not achievable.
- Specimens shall be divided into amounts required for one testing and stored at −20℃ in a freezer so as to avoid from thawing repeatedly.
- Specimens that are no longer needed shall be put in a container for liquids and disposed as liquid medical waste.

7. PRE-TEST PREPARATIONS
- Reagents shall be stored at −20℃ and be avoided from repeated freezing and thawing.
- Reagents shall be used after completely thawed and well mixed.
- Since the positive control can be degraded, it is recommended to be aliquoted into small volumes.
- This kit was validated on ABI StepOne Plus system and compatible with standard RealTime-PCR machines such as ABI 7000, 7300,7500, Roche 480, MX3000P, MX3005P, Rotorgene TM6000, Icycler IQTM4/5, Bio-Rad CFX96, LongGene Q2000B and OptiGene Genie II.

8. SPECIMEN PRETREATMENT
While it is possible to use various ways and kits adopted in laboratories to extract nucleic acids and apply on this product, it is recommended that Quick DNA/RNA Extraction Solution (Sugenomics Biotechnology, Cat#: NA04A100) shall be used for nucleic acid extraction and users shall follow the protocol included in the kit handbook. After being extracted, nucleic acids shall be stored at −20±2℃ in a freezer and shall be divided into small.

9. ISOTHERMAL LAMP PCR
9.1) Isothermal LAMP PCR Solution Preparation (on ice)
a) Thaw kit components at room temperature.
b) Mix thoroughly to ensure homogeneity, centrifuge briefly and then put on the ice.
c) Prepare the reaction mix based on the table below.
d) It is recommended to use positive and negative controls for each test.
e) Spin down all reaction mixture and move to the LAMP PCR step.

Components	Sample DNA	Positive Control	Negative Control
LCDV LAMP Master Mix	17.5 µl	17.5 µl	17.5 µl
LCDV LAMP Primer Mix	2.5 µl	2.5 µl	2.5 µl
LCDV LAMP Positive Control	-	5 µl	-
LCDV LAMP Negative Control	-	-	5 µl
LCDV LAMP Sample DNA	5 µl	-	-
Total Volume	**25 µl**	**25 µl**	**25 µl**

9.2) Reaction Set Up Conditions
- Set up the channels of the real-time PCR device according to the table below.

Gene	Channels (Dyes)
***Lymphocystis Disease Virus, MCP* gene**	FAM or SYBRGreen

- For Applied Biosystems ABI (QS5, 7500 and StepOne etc) real-time PCR instruments, set to "passive reference" dye as "none".
- Set up isothermal LAMP PCR temperature, time, and cycling conditions according to the table below.

Steps	Temp.	Time	Cycles
Amplification	70 ℃	30 sec (Reading at the end of each cycle)	70*

***70 cycles take for 35 minutes.**

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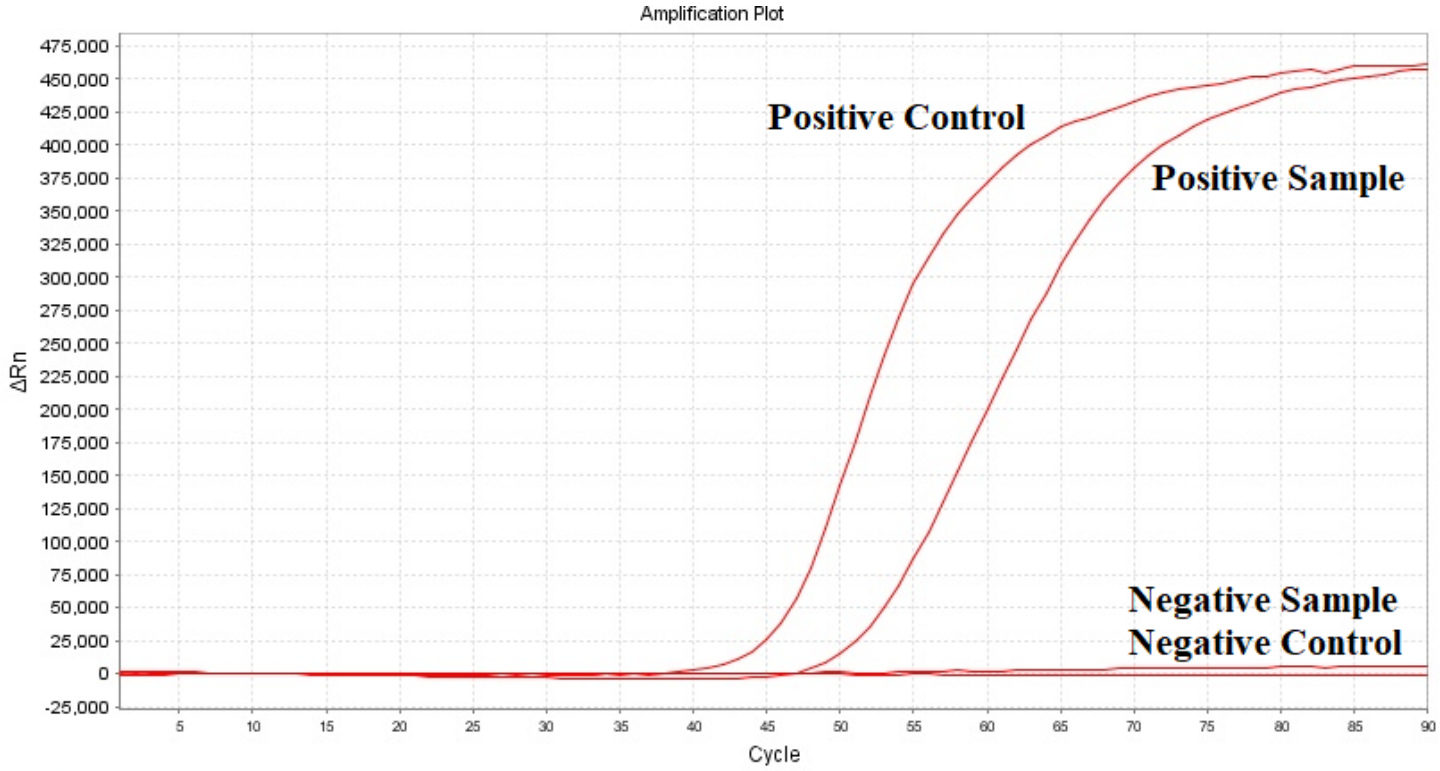
10. EVALUATIONS of RESULTS

Analyze the amplification of the samples, positive and negative controls for any increase. If the fluorescence increases in Positive Control but doesn't in Negative Control, amplification reaction is proceeding properly (Fig 1). If any other situation occurs, however, amplification reaction may be proceeding in a wrong way. In such a case, re-test affected samples from reagent preparation.

Figure1. Amplification plots for positive sample, negative sample, and positive and negative controls. Positive samples were detected at 28-42th cycles (14-21 minutes) according to the level of sample nucleic acid concentration. ABI StepOne Plus system was used.



11. PRODUCT SPECIFICITY

1. Minimum detection limit is 50 viral genomes equivalent per test.
2. Negative Sample (concentration: 0 genome/test)
3. Positive Sample contains equivalent to 1000 genomes/test

12. LIMITATIONS

1. Test result is for the reference only in clinical practice, it cannot be the sole evidence for diagnosis.
2. Negative results can be caused by low quality of nucleic acid extraction, improper storage conditions and storage period, and inhibitors in the specimen, nucleic acid degradation, etc.
3. False negative or false positive results are likely to be caused by inappropriate collecting, transportation and handling of specimens, or unsuitable experiment operation and environment.

SUGENOMICS Biotechnology

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