



Ready To Use PCR Reagents

FELINE LEUKEMIA VIRUS

Cat. No. 60FLV100

INSTRUCTION MANUAL

I. Intended Use

FLV PCR Ready to Use PCR Reagents are intended for Feline Leukemia Virus amplifications. All reagents are ready to use for a successful amplification, from DNA extraction to obtaining PCR products suitable for loading onto Agarose gel.

II. General Information

Each package contains **Rapid One Step Extraction Buffer** (Tube A), which is intended for use with fresh or dry blood samples. The extraction step yields appropriate amount of crude DNA needed for a successful amplification of **FLV** via PCR. No purification is needed! Tubes **B**, **C** and **D** are the components for subsequent use in PCR amplification. Tube **B** contains **FLV-PCR mix**, Tube **C** contains **FLV Activation Buffer** and Tube **D** contains the **Positive Control**. The Extraction Buffer (Tube **A**) also serves as **Negative Control**. Also included are **Tissue/Swab Extraction Buffer** (Tube **E**) and **Tissue/Swab Neutralization Buffer** (Tube **F**). Each PCR set up should include 3 reaction vials; each vial should be added with: Each PCR set up should include 3 reaction vials, into which are added **5µl FLV-PCR mix**, **10µl FLV Activation Buffer** and **5µl DNA product of the Extraction step / Positive Control/ Negative Control**. Following the addition and mixing of all the above ingredients, the reaction vials are placed in thermal cycler for amplification according to the program detailed in the Step by Step chapter (see section VIII). At the end of the program the product should be visualized on 1.5% Agarose gel, yielding a **435bp** band.

III. Description Of The Disease

Feline Leukemia Virus (FeLV) is a γ -retrovirus naturally occurring worldwide in domestic cats and some related small felids. FeLV infection is a contagious disease, transmitted primarily horizontally via oronasal route; vertical transmission may occur as well. Infection may lead to fatal neoplasia, degenerative diseases of the hematopoietic system and immunodeficiency. Vaccines against FeLV infection have been commercially available for almost two decades. Yet cats believed to be immune to FeLV infection were found to turn provirus-positive after virus exposure. Moreover, efficacious considered FeLV vaccines were found unable to prevent provirus-integration and minimal viral replication. FeLV provirus was found to persist for years leading to reoccurrence of viremia and disease development.

IV. Diagnosis Of The Disease

PCR and RT-PCR assays were demonstrated to be highly sensitive and specific. Their results yield a more sensitive measure for FeLV exposure than antigen detection, virus isolation or immunofluorescence assays. Consequently, using PCR assays to identify FeLV exposed cats, particularly in catteries and to investigate obscure clinical problems that may be FeLV-associated, is recommend. FeLV is frequently found in whole blood samples, spleen, liver and bone marrow.

V. Contents (Sufficient for 48 tests)

Tube A	Rapid One Step Blood Extraction Buffer
Tube B	FLV-PCR mix (Green cap)
Tube C	Specific FLV Activation Buffer (Blue cap)
Tube D	Specific FLV Positive Control (Red cap)
Tube E	Tissue/Swab Extraction Buffer
Tube F	Tissue/Swab Neutralization Buffer
	FLV Instruction Manual

VI. Essentials Not Included

- RNAase free PCR reaction vials.
- PCR Thermo-Cycler.
- 5-10µ, 100µl Pipettes and filter tips.
- Micro-centrifuge.
- Heating bath or heating block.
- Agarose, DNA size marker.
- Microwave for Agarose casting.
- Horizontal Mini-Electrophoresis chamber, Comb and power pack.
- TBE /TAE Buffer and Ethidium Bromide (EB).
- UV Transilluminator (254nm for EB).
- A pair of sterile scissors.
- A cutter (for swab application).

VII. Storage And Handling

- Store at 4°C for 6 months or at -20°C for two years.
- Use gloves and maintain clean working conditions.
- Avoid spillage and cross contamination of solutions.
- Change tips between reagents and between reaction vials.
- Disinfect scissors before and after each cutting of blood filters.
- Do not mix reagents from different batches.
- Always treat samples with precaution, and dispose as biological material.
- Remember that Ethidium Bromide is hazardous, and use the UV transilluminator carefully.
- It is recommended to incinerate the contents after use.

Kit developed by Karnieli Ltd.

60FLV148-30.09.09

VIII. Step By Step Protocol

Blood Extraction:

(1) Into an empty clean vial, add **100µl of Rapid OneStep Blood Extraction Buffer (Tube A)** for **every 5µl** of fresh blood sample or approximately 3/5 mm² piece of Whatman/tissue paper soaked with blood. Make sure the piece of paper is submerged underneath the extraction buffer.

(2) Incubate samples at **50°C** for **10 minutes** followed by a subsequent **95°C** for additional **10 minutes**.

(3) Centrifuge sample at **>10,000 rpm** for **1 minute** to allow the paper and cell debris to pellet. The extracted DNA product is in the liquid phase, ready to be used for PCR.

Tissue/Swab Extraction:

(1) Into a clean 1.5 ml vial add **300µl of Tissue/Swab Extraction Buffer (Tube E)**.

(2) When using tissue sample, cut a 3 mm² from the fresh or frozen tissue and add it to the 1.5 ml vial containing **300µl of Tissue/Swab Extraction Buffer**.

(3) Incubate the tissue within buffer **E** for **10 minutes at 95°C**.

(4) Add **300µl of Tissue/Swab Neutralization Buffer (Tube F)** and the product will be ready for PCR use.

Extracted DNA product (of any source)* may be applied immediately for PCR or stored for a few days at 4°C / several months at -20°C. Please mark the vial properly for future identification.

* Note: **The reagents have been adjusted for use with crude DNA extraction to enable better sensitivity (with less handling).**

PCR Procedure:

(1) Into a clean reaction vial add: **5µl FLV-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific FLV-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.

(2) Into a second clean reaction vial add **5µl FLV-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific FLV Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.

(3) Into a third clean reaction vial add **5µl FLV-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific FLV Activation Buffer (Tube C)**. Mark this vial as **Negative Control** reaction.

(4) Gently mix each reaction vial (do not vortex!) and place in the thermal cycler for amplification.

PCR Program:

A. 95°C for 2 minutes

38 cycles of:

B. 94°C for 30 seconds

C. 56°C for 30 seconds

D. 72°C for 30 seconds

End cycles

E. 72°C for 2 minutes

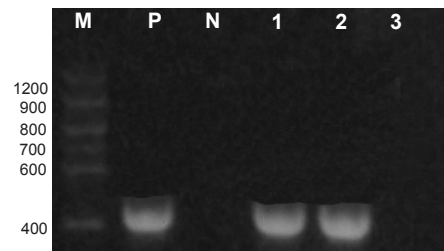
F. Stop at 8°C

(5) If not used immediately, store PCR products at 4°C until application on Agarose.

IX. Reading And Interpreting The Results

- Visualize PCR products on 1.5% Agarose gel, along with a size marker (see Fig. 1).
- Mark each well and load the whole content of each reaction vial into the relevant wells.
- The Positive Control should yield a single band at **435bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **435bp**.

Fig. 1 - Visualization of the PCR product.



Lanes: M Size Marker, P Positive Control, N Negative Control
Lanes 1 - 3 are test samples of which 1 and 2 are positive for FLV.

X. Limitations And Troubleshooting

- For *in vitro* use only. Do not use internally or externally in humans or animals.
- A false positive result may occur, even if precaution has been taken. To eliminate inconclusive results, always use the Negative Control in each PCR set.
- To avoid false positive interpretation, check vaccination schedules. PCR may be positive 2-6 weeks post vaccination.

XI. References

- Hofmann-Lehmann R et al. (2001) Feline leukaemia provirus load during the course of experimental infection and in naturally infected cats. J. Gen. Virol. 82: 1589–1596.
- Hofmann-Lehmann R et al. (2006) Reassessment of feline leukaemia virus (FeLV) vaccines with novel sensitive molecular assays. Vaccine 24: 1087–1094.
- Hofmann-Lehmann R et al. (2008) How molecular methods change our views of FeLV infection and vaccination. Vet Immunol Immunopathol. 15;123(1-2):119-23. Epub 2008 Jan 19.
- Torres A N et al. (2005) Re-examination of feline leukemia virus:host relationships using real-time PCR. Virology 332: 272–283.

For further information and assistance please contact your local distributor or Biogal Galed Labs. Directly by e-mail: info@biogal.co.il or by tel: 972-4-9898605 / fax: 972-4-9898690.