

FELINE PANLEUKOPENIA VIRUS

Cat. No. 60FPV100
INSTRUCTION MANUAL

I. Intended Use

FPV Ready to Use PCR Reagents are intended for Feline Panleukopenia 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 **FPV** via PCR. No purification is needed! Tubes **B**, **C**, and **D** are the components for subsequent use in PCR amplification. Tube **B** contains **FPV-PCR mix**, Tube **C** contains **FPV 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 FPV-PCR mix**, **10µl FPV 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 **340bp** band.

III. Description Of The Disease

Feline Panleukopenia virus (FPV), which is classified in the parvovirus group, is a common viral organism infecting domestic cats and some other Felidae (including tigers, panthers, and leopards) as well as species of the families Mustelidae, Procyonidae, and Viverridae including raccoon, ringtailed cat and mink. The virus causes feline panleukopenia, also known as feline infectious enteritis or feline "distemper". It is highly contagious and associated with significant morbidity and mortality. Not all cats infected will develop clinical signs, but if they do, the disease can be peracute and severe (mortality rate up to 100%) often without diarrhea, or acute (25- 90% mortality) with fever, anorexia and vomiting sometimes followed by diarrhea. The virus is highly resistant and can survive for up to 1 year in infected organic material. Moreover, FPV can be transmitted to susceptible cats through clothes, shoes or transport cages.

IV. Diagnosis Of The Disease

Clinical signs in cats are variable and leucopenia is not always present at the time of presentation. Early detection of the virus with reliable test systems is crucial in order to separate infected cats (especially in veterinary hospitals with other severely ill and immunocompromised animals), to treat the patients aggressively, and to inform the owners about prognosis and potential risks for other household cats. Recommended confirmation tests to diagnose parvovirus infection in vivo are electron microscopy of fecal samples or polymerase chain reaction (PCR) of feces, blood, or infected tissues. The PCR is highly sensitive and specific, providing a reliable and effective test.

V. Contents (Sufficient for 48 tests)

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

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)**. Proceed to steps **2a** or **2b**.
- (2a) When using swab: carefully cut the agar-free swab close to its cotton edge and insert it into the vial. The swab should fit entirely inside the vial, must be covered with buffer and the cap should close easily.
- (2b) When using tissue: 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 swab or 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 FPV-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific FPV-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl FPV-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific FPV Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl FPV-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific FPV 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:

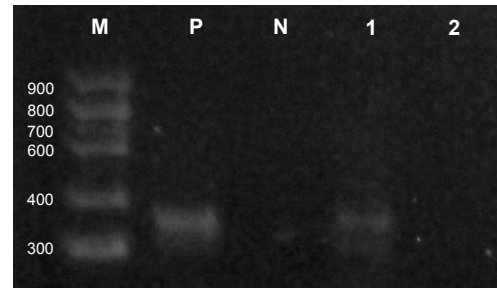
- 95°C for 2 minutes**
- 38 cycles of:**
- 94°C for 30 seconds**
 - 56°C for 30 seconds**
 - 72°C for 30 seconds**
- End cycles**
- 72°C for 2 minutes**
 - Stop at 8°C**

- (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 **340bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **340bp**.

Fig. 1 - Visualization of the PCR product.



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

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.
- Cats may be infected by any canine parvovirus, (exhibiting symptoms similar to FPLV) which would not be detected by the FPLV PCR.

XI. References

- Esfandiari J & Klingeborn B (2000) A comparative study of a new rapid and one-step test for the detection of parvovirus in faeces from dogs, cats and mink. Journal of Veterinary Medicine B 47, 145e153.
- Neuerer FF et al. (2008) Comparison of different in-house test systems to detect parvovirus in faeces of cats. J Feline Med Surg.10(3):247-51. Epub 2008 Feb 20
- Schatzberg SJ (2003) Polymerase chain reaction (PCR) amplification of parvoviral DNA from the brains of dogs and cats with cerebellar hypoplasia. Journal of Veterinary Internal Medicine 17, 538e544.
- Scott FW (1987) Viral diseases. Panleucopenia. In: Holzworth J (ed), Diseases of the Cat: Medicine and Surgery. Philadelphia: WB Saunders, pp. 182e193.

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.