



# Ready To Use PCR Reagents

## FELINE HERPES VIRUS

Cat. No. 60FHV100

### INSTRUCTION MANUAL

#### I. Intended Use

**FHV Ready to Use PCR Reagents** are intended for Feline Herpes Virus (FHV) 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 Feline Herpes Virus via PCR. No purification is needed! Tubes B, C and D are the components for subsequent use in PCR amplification. Tube B contains **FHV-PCR mix**, Tube C contains **FHV 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: **5µl FHV-PCR mix**, **10µl FHV 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 **360bp** band.

#### III. Description Of The Disease

Feline herpesvirus-1(FHV-1) is the most frequent cause of conjunctivitis and keratitis in domestic cats. The virus is ubiquitous in domestic cat populations. FHV-1 is also the most studied infectious cause of ocular surface disease in cats. Cats infected with FHV-1 can present conjunctival, corneal or a combination of conjunctival and corneal signs; they may also present systemic illness or upper respiratory signs. FHV-1-associated ocular diseases include chronic conjunctivitis, symblepharon, keratoconjunctivitis sicca, eosinophilic keratitis, stromal keratitis and corneal sequestrum. Mortality rates of up to 70% have been reported in infected kittens.

#### IV. Diagnosis Of The Disease

Acute FHV-1 infection is usually diagnosed based on ocular and respiratory clinical signs; laboratory testing is necessary for diagnosis and verification. FHV-1 has been isolated from eosinophilic keratitis specimens. PCR tests were reported to be more sensitive than viral isolation or Fluorescent antibody testing in cats with conjunctivitis or with upper respiratory tract disease and conjunctivitis. FHV-1 DNA has been detected in numerous ocular tissues using PCR technology. Final diagnosis should include other parameters such as clinical signs into considerations.

#### V. Contents (Sufficient for 48 tests)

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

#### 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.

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## 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 mm<sup>2</sup> 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 mm<sup>2</sup> 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 kit 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 FHV-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific FHV-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.

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

(3) Into a third clean reaction vial add **5µl FHV-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific FHV 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 3 minutes**

**A. 2x**

**94°C for 30 seconds**

**58°C for 30 seconds**

**72°C for 30 seconds**

**B. 2x**

**94°C for 30 seconds**

**56°C for 30 seconds**

**72°C for 30 seconds**

**C. 2x**

**94°C for 30 seconds**

**54°C for 30 seconds**

**72°C for 30 seconds**

**D. 35x**

**94°C for 30 seconds**

**53°C for 30 seconds**

**72°C for 30 seconds**

**E. 72°C for 2 minutes.**

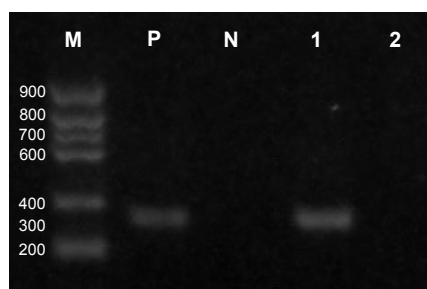
**F. Stop at 8°C**

(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 **360bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **360bp**.

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 FHV.

## 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. Avoid testing during 2-6 weeks post FHV vaccination.

## XI. References

- Andrew SE (2001) Ocular manifestations of feline herpesvirus. J Feline Med Surg.3(1):9-16. Erratum in: J Feline Med Surg 3(2):115.
- Glaze MB & Gelatt KN (1999) Feline ophthalmology. In: Veterinary Ophthalmology (3rd edn). Gelatt KN (ed.). Lippincott, Williams & Wilkins, Philadelphia, pp. 997–1052.
- Lutz H et al. (1999) The role of polymerase chain reaction and its newer developments in feline medicine. Journal of Feline Medicine and Surgery 1, 89–100.
- Stiles J et al. (1997) Comparison of nested polymerase chain reaction, virus isolation, and fluorescent antibody testing for identifying feline herpesvirus in cats with conjunctivitis. American Journal of Veterinary Research 58, 804–807.

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.