



# Ready To Use PCR Reagents

## FELINE CALICIVIRUS

Cat. No. 60FCV100  
INSTRUCTION MANUAL

### I. Intended Use

**FCV Ready to Use PCR Reagents** are intended for Feline Calicivirus amplifications. All reagents are ready to use for a successful amplification of the viral cDNA and obtaining PCR products suitable for loading onto Agarose gel.

### II. General Information

Each package contains **cDNA Diluting Buffer** (Tube **A**), which is intended to dilute the cDNA prior to the PCR amplification. The remaining 3 tubes are the components for subsequent use in PCR amplification. Tube **B** contains **FCV-PCR mix**, Tube **C** contains **FCV Activation Buffer** and Tube **D** contains the **Positive Control**. The Diluting Buffer (Tube **A**) also serves as **Negative Control**. Each PCR set up should include 3 reaction vials; each vial should be added with: **5µl FCV-PCR mix**, **10µl FCV Activation Buffer** and **5µl DNA product of the Diluting 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 **530bp** band.

### III. Description Of The Disease

Feline Calicivirus (FCV), member of the family Caliciviridae, genus Vesivirus is a major cause of acute and chronic upper respiratory diseases in cats. Commonly seen clinical signs include rhinitis, conjunctivitis, stomatitis, gingivitis and glossitis. Less often seen are pneumonia, fever, abortion, cystitis and limping. After resolution of the infection, apparently healthy cats may shed virus for several months or even years. Prevalence rates of FCV in cats without clinical symptoms vary between 2.5 and 25%. These asymptomatic carrier cats are believed to contribute to the epidemiology of FCV infection and disease. Recently, virulent FCV strains associated with a hemorrhagic like fever and mortality up to 60% in adult cats have been described.

### IV. Diagnosis Of The Disease

Diagnosis is commonly based on clinical signs alone such as predominantly oral ulceration. Serology is generally not helpful in the diagnosis of FCV infection due to widespread antibody from vaccination. Confirmation of diagnosis may be done by PCR test. The FCV genome consists of a single-stranded positive-sense RNA with a size of 7.7 kb and contains three open reading frames (ORF). ORF1 encodes the various non-structural proteins, ORF 2 encodes the capsid protein (VP1) and ORF 3 encodes second minor structural protein (VP2). RNA is extracted from either oropharyngeal swabs, ideally in the first week of illness (sent chilled to the laboratory within 24 hours), or from blood samples.

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

Tube <b>A</b>	<b>Diluting Buffer</b>
Tube <b>B</b>	<b>FCV-PCR mix (Green cap)</b>
Tube <b>C</b>	<b>Specific FCV Activation Buffer (Blue cap)</b>
Tube <b>D</b>	<b>Specific FCV Positive Control (Red cap)</b>
	<b>FCV Instruction Manual</b>

### VI. Essentials Not Included

RNA Extraction kit.  
cDNA Random Priming/Synthesis kit.  
RNAase free PCR reaction vials.  
PCR Thermo-Cycler.  
5-10µ, 100µl Pipettes and RNase free 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).

### 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

(1) Extract total RNA from blood or oropharyngeal swab using one of the commercial available **RNA extraction kits** following the vendor's protocol, including purity and quality validation. If not used immediately, store extract at -70°C.

(2) Prepare **cDNA** using **random priming** from one of the commercial cDNA kits according to the vendor's instructions. If not used immediately, store extract at -70°C.

(3) If RNA extraction yielded more than 1µg of total RNA per 20µl cDNA preparation, dilute the cDNA in 1:1 V/V with the Diluting buffer (Tube A).

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

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

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

(7) Gently mix each reaction vial and place in the thermal cyclor for amplification.

### PCR Program:

A. 95°C for 2 minutes

38 cycles of:

B. 94°C for 30 seconds

C. 53°C for 30 seconds

D. 72°C for 30 seconds

End cycles

E. 72°C for 2 minutes

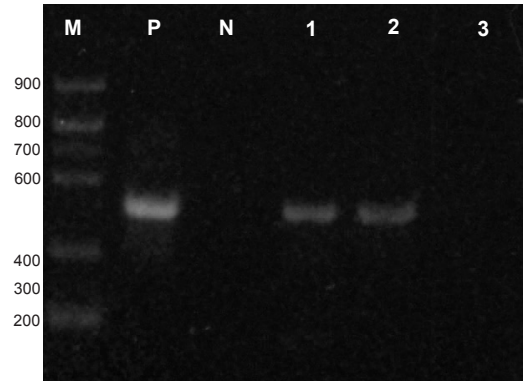
F. Stop at 8°C

(8) 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 **530bp**.
- No band should be detected at the Negative Control lane.
- The expected product should yield a **530bp** band.

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 FCV.

## 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 FCV vaccination.
- Due to high recombination rate some variants may occur in band size.

## XI. References

- Coutts AJ et al. (1994) Isolation of feline respiratory viruses from clinically healthy cats at UK shows. *Vet. Rec.* 135: 555–556.
- Green KY et al. (2002) Isolation of enzymatically active replication complexes from feline calicivirus infected cells. *J. Virol.* 76: 8582–8595.
- Hurley KF et al. (2004) An outbreak of virulent systemic feline calicivirus disease. *J. Am. Vet. Med. Assoc.* 224: 241–249.
- Pedersen NC et al. (2000) An isolated epizootic of hemorrhagic-like fever in cats caused by a novel and highly virulent strain of feline calicivirus. *Vet. Microbiol.* 73:281–300.
- Schorr-Evans EM et al. (2003) An epizootic of highly virulent feline calicivirus disease in a hospital setting in New England. *J. Feline Med. Surg.* 5: 217–226
- Wilhelm S (2006) Real-time reverse transcription polymerase chain reaction assay to detect a broad range of feline calicivirus isolates. *J Virol Methods.* 133(1):105-8. Epub 2005 Nov 2.

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.