



Ready To Use PCR Reagents

FELINE CORONAVIRUS

Cat. No. 60FCR100
INSTRUCTION MANUAL

I. Intended Use

FCR Ready to Use PCR Reagents are intended for Feline Coronavirus amplifications. All reagents are ready to use for a successful amplification of 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 **FCR-PCR mix**, Tube C contains **FCR 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 FCR-PCR mix**, **10µl FCR 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 **177/270bp** band.

III. Description Of The Disease

Feline Coronavirus (FCoV) IS known to have two biotypes: Feline Enteric Coronavirus (FECV) and Feline Infectious Peritonitis Virus (FIPV). FECV is extremely widespread in cat populations, often being asymptomatic or causing only mild enteric infections. Molecular studies have suggested that random mutations in the FECV genome induce appearance of virulent FIPV variants in infected cats; the presence of these variants coupled with an inadequate immune response leads to the development of a **fatal immune-mediated clinical disease**. Factors recognized to be important in the epidemiology and development of FIP are: age at the time of viral exposure, the physical condition of the cat, genetic disposition, stress, presence of concurrent disease (such as feline leukaemia virus and feline immunodeficiency virus), multi-cat households, dose and strain of FCoV, pre-existing FCoV antibodies and level of cell-mediated immunity response.

IV. Diagnosis Of The Disease

Antibodies against FCoV are found in 80–90% of the animals living in catteries or multiple-cat households and in up to 50% of solitary cats; however, only 1–5% of the sero-positive cats eventually come down with FIP. The advantage of PCR is the ability to identify the presence of the virus in the sample. The PCR test has fewer FCoV positive results in healthy cats making it a preferred technique for screening. An important event in FIP pathogenesis is the infection of monocytes and macrophages. Whole blood samples are the easiest sampling material. In FIP positive cats, the virus can be found in fluids from the peritoneum as well. Positive PCR result alone does not allow a definite diagnosis but is supportive to the clinical symptoms; where as a negative result is defendant for ruling out FIP.

V. Contents (Sufficient for 48 tests)

Tube A	Diluting Buffer
Tube B	FCR-PCR mix (Green cap)
Tube C	Specific FCR Activation Buffer (Blue cap)
Tube D	Specific FCR Positive Control (Red cap)
	FCR Instruction Manual

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 FCR-PCR mix** (Tube B), **5µl of the Diluted cDNA product** and **10µl of the specific FCR-Activation Buffer** (Tube C). Mark each reaction vial properly to avoid mistakes.

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

(6) Into a third clean reaction vial add **5µl FCR-PCR mix** (Tube B), **5µl of the Diluting Buffer** (Tube A) and **10µl of the specific FCR 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**

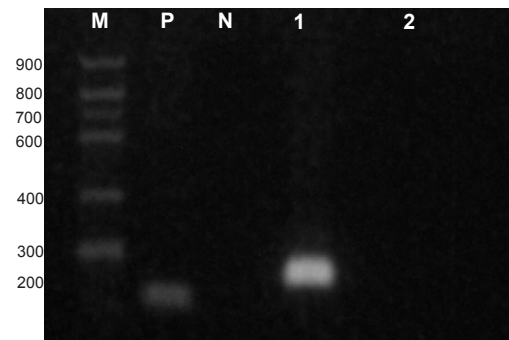
F. **Stop at 8°C**

(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 **177bp**.
- No band should be detected at the Negative Control lane.
- The expected product should yield a **177/270bp** band.

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 FCR.

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 FIP vaccination.
- PCR results can only be interpreted in correlation with clinical symptoms.

XI. References

- Gunn-Moore DA et al. (1998) Detection of feline coronaviruses by culture and reverse transcriptase-polymerase chain reaction of blood samples from healthy cats and cats with clinical feline infectious peritonitis. *Vet. Microbiol.* 62: 193–205.
- Lai MMC & Cavanagh D (1997) The molecular biology of coronaviruses. *Adv. Virus. Res.* 48: 1–100.
- Simons FA et al. (2005) A mRNA PCR for the diagnosis of feline infectious peritonitis. *J Virol Methods.* 124(1-2):111-6. Epub 2004 Dec 21
- Stoddart CA & Scott FW (1989) Intrinsic resistance of feline peritoneal macrophages to coronavirus infection correlates with *in vivo* virulence. *J. Virol.* 73 (1): 436–440.

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.