Ready To Use PCR Reagents

Feline *Chlamidophila felis*

Cat. No. 60FCS100

INSTRUCTION MANUAL

I. Intended Use

FCS Ready to Use PCR Reagents are intended for Feline *Chlamydophila felis* 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 FCS via PCR. No purification is needed!

Tubes **B**, **C** and **D** are the components for subsequent use in PCR amplification. Tube **B** contains **FCS-PCR mix**, Tube **C** contains **FCS 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 FCS-PCR mix**, **10µl FCS 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 **480bp** band.

III. Description Of The Disease

Chlamydophila felis (previously *Chlamydia psittaci* var. felis) is an obligate, intracellular bacteria, with cell walls resembling those of Gram-negative bacteria. The chlamydial developmental cycle involves an alternation between the predominantly extracellular, infectious elementary body (EB), measuring 0.3 µm in diameter, and the intracellular, metabolically active reticulate body (RB), measuring 0.5 to 1.5µm in diameter. C. felis is primarily a conjunctival pathogen, capable of causing acute to chronic conjunctivitis, with blepharospasm, chemosis, congestion and a serous to mucopurulent ocular discharge. Transient fever, inappetence and weight loss may occur shortly after infection, although most cats apparently remain well and continue to eat. Clinical signs improve after a few weeks but mild conjunctivitis often persists for months.

IV. Diagnosis Of The Disease

Chlamydophila infection needs to be differentiated from other infectious and noninfectious causes of feline conjunctivitis. Because it is not possible to differentiate infections caused by FCV, FHV-1 and *C. felis* solely based on clinical signs, an accurate diagnosis is required. Diagnostic PCR assays are more rapid and less expensive than traditional techniques such as culturing. PCR has good sensitivity and specificity. Samples collected from the conjunctival sac using a saline-moistened cotton swab are preferable.

V. Contents (Sufficient for 48 tests)

Tube **A**	**Rapid One Step Blood Extraction Buffer**
Tube **B**	**FCS-PCR mix (Green cap)**
Tube **C**	**Specific FCS Activation Buffer (Blue cap)**
Tube **D**	**Specific FCS 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 Ethydium 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 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^2 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) 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.

(3) Incubate the swab 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 FCS-PCR mix** (Tube **B**), **5µl** of the **Extracted DNA product** and **10µl** of the specific **FCS-Activation Buffer** (Tube **C**). Mark each reaction vial properly to avoid mistakes.

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

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

(4) Gently mix each reaction vial and place in the thermal cycler for amplification.

PCR Program:

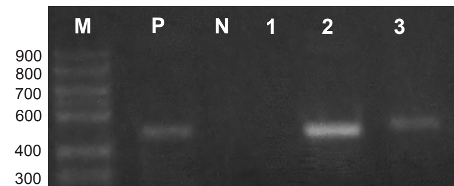
A. 95°C for 2 minutes
38 cycles of:
B. 94°C for 30 seconds
C. 56°C for 30 seconds
D. 72°C for 30 seconds
End cycles
E. 72°C for 2 minutes
F. Stop at 8°C

(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 **480bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **480bp**.

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 2 and 3 are positive for FCS.

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 *Chlamydophila* vaccination.

XI. References

- Di Francesco A (2004). Prevalence of *Chlamydophila felis* by PCR among healthy pet cats in Italy. New Microbiol 27:199-201.
- Rampazzo A et al. (2003) Prevalence of *Chlamydophila felis* and feline herpesvirus 1 in cats with conjunctivitis in northern Italy. J Vet Intern Med 17:799-807.
- Sykes JE et al. (1999) Prevalence of feline *Chlamydia psittaci* and feline herpesvirus 1 in cats with upper respiratory tract disease. J Vet Intern Med 13:153-162.
- Sykes JE (2005) Feline chlamydiosis. Clin Tech Small Anim Pract. 20(2):129-34.
- Von Bomhard W et al. (2003) Detection of novel chlamydiae in cats with ocular disease. Am J Vet Res 64:1421-1428,

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.