



“Ready To Use PCR Reagents

PSITTACINE BEAK AND FEATHER DISEASE VIRUS

Cat. No. 60ABF100

INSTRUCTION MANUAL

I. Intended Use

PBFDV Ready to Use PCR Reagents are intended for Beak and Feather Disease 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 PBFDV via PCR. No purification is needed! The remaining 3 tubes are the components for subsequent use in PCR amplification. Tube B contains **PBFDV-PCR mix**, Tube C contains **PBFDV 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 PBFDV-PCR mix**, **10µl PBFDV 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 **700bp** band.

III. Description Of The Disease

Psittacine Beak and Feather Disease (PBFD) is a viral disease affecting all Old and New World Parrots (*Psittacini Hookbills*). The virus, a Circoviridae member, attacks the feather follicles, beak and claws-growing cells, causing progressive feather malformation and necrosis. Later, the feathers develop constrictions in feather shafts, development ceases, until all feather growth stops. The beak and claws are affected by overgrowth, malformation and necrosis. Cracking and peeling of outer layers can lead to secondary infections. Necrosis of inner layers of the beak may cause its breaking and inability to feed. The disease has also a **general immunosuppressive effect on the bird**, clearing path for secondary systemic infections, which are usually the cause of death. Birds with PBFD may have normal feathers, so the PCR test is the most efficient method available for early detection of the virus.

IV. Diagnosis Of The Disease

The most efficient way to diagnose the presence of PBFDV is by PCR amplification of the virus DNA in both blood and feather samples.

The PBFDV has a very unique circular signal strand DNA genome, built out of about 2000bp. The PCR test uses a unique primer sequences which identify and amplify the virus. These sequences can amplify the known PBFDV variants but will not amplify close viruses such as the porcine Circovirus. Apart from the **700bp** band, the ABF PCR kit may detect known variants of **500** or **750bp** bands. A positive amplification means that the virus DNA is present in the tested sample.

V. Contents (Sufficient for 48 tests)

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

VI. Essentials Not Included

RNAase free PCR reaction vials.
PCR Thermo-Cycler.
5-10µl, 100µl Pipettes and filter tips.
Micro-centrifuge.
Vortex.
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 month 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 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.

Feather Extraction:

- (1) Cut de lower portion (root) of freshly plucked feather (5mm) and insert it into a clean 1.5 ml vial.
- (2) Add **100µl of Tissue/Swab Extraction Buffer (Tube E)**. Make sure the sample is submerged underneath.
- (3) Incubate sample at **95°** for **10 minutes**.
- (4) Centrifuge sample at **>10,000 rpm** for **1 minute** and transfer supernatant to a new clean 1.5 ml vial.
- (5) Add **100µl of Tissue/Swab Neutralization Buffer (Tube F)**, briefly vortex mixture 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 PBFDV-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific PBFDV-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl PBFDV-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific PBFDV Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl PBFDV-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific PBFDV 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:

A. 95°C for 2 minutes

38 cycles of:

B. 94°C for 30 seconds

C. 58°C for 30 seconds

D. 72°C for 30 seconds

End cycles

E. 72°C for 2 minutes

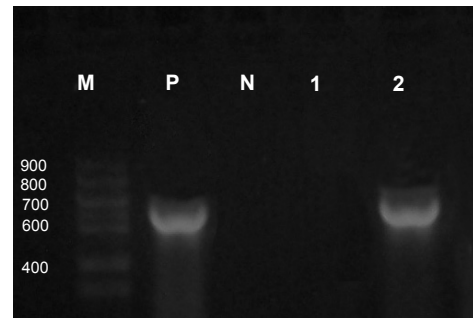
F. Stop at 8°C

- (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 **700bp**.
- No band should be detected at the Negative Control lane.
- In case of a positive result a single band should at **700bp** should be visible in the sample lane.

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 2 is positive for PBFDV.

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.

XI. References

- Bendhiem U et al. (2006) Prevalence of Psittacine Circovirus in Israel. IJOVM, 61 (1) 12-15.
- Heath L et al. (2004) Evidence of unique genotypes of beak and feather disease virus in southern Africa. J Virol.78(17):9277-84.
- Khalesi Betal. (2005) A comparison of haemagglutination, haemagglutination inhibition and PCR for the detection of psittacine beak and feather disease virus infection and a comparison of isolates obtained from lorrids. J Gen Virol.86(Pt 11):3039-46.
- de Kloet E and de Kloet SR (2004) Analysis of the beak and feather disease viral genome indicates the existence of several genotypes which have a complex psittacine host specificity. Arch Virol.149(12):2393-412. Epub 2004 Jul 15.
- Shearer PL et al. (2008) Beak and feather disease virus infection in cockatiels (*Nymphicus hollandicus*). Avian Pathol. 37(1):75-81.
- Ypelaar I et al. (1999) A universal polymerase chain reaction for the detection of psittacine beak and feather disease virus. Vet Microbiol. 16;68(1-2):141-8.

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.