



Ready To Use PCR Reagents

AVIAN POLYOMA VIRUS

Cat. No. 60APV100
INSTRUCTION MANUAL

I. Intended Use

APV Ready to Use PCR Reagents are intended for Avian Polyoma Virus amplifications. All reagents are ready to use for a successful amplification, from DNA extraction to obtaining PCR products suitable for loading onto the 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 APV via PCR. No purification is needed! Tubes B, C, and D are the components for subsequent use in PCR amplification. Tube B contains **APV-PCR mix**, Tube C contains **APV 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 APV-PCR mix**, **10µl APV 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 **440bp** band.

III. Description Of The Disease

Avian Polyoma Virus (APV) was first described in 1981 as the etiologic agent of budgerigar fledgling disease, a devastating disease of young budgerigars, and was subsequently designated budgerigar fledgling disease virus. The disease is characterized by hepatitis, ascites, and hydropericardium as the main clinical symptoms and by mortality rate of up to 100% in fledglings. Young budgerigars which survive the infection, develop a chronic course of disease with feather disorders as the main clinical symptom. APV infections have also been detected in other parrot species with similar clinical signs, which vary in their susceptibility and severity depending on the species. A distinct disease course found in older parrots is characterized by sudden death accompanied by a membranous glomerulopathy. Birds of other families such as finches, buzzards and falcons, also seem to be susceptible to infection with APV.

APV has been found in many countries, suggesting a worldwide distribution.

IV. Diagnosis Of The Disease

The genome of all members of the family Polyomaviridae consists of one circular double stranded DNA molecule, approximately 5,000 bp in size. The APV genome is unique, allowing efficient PCR detection and determination. When testing individual birds, a whole blood sample in conjunction with a separate cloacal swab is recommended when possible. Post mortem samples of liver, spleen or kidney tissue in a sterile container and postmortem swabs may also be submitted. Environmental testing using swabs of aviaries, countertops, fans, air-filters, nest-boxes etc. is extremely effective in determining the presence of Polyoma DNA in the environment.

V. Contents (Sufficient for 48 tests)

Tube A	Rapid One Step Blood Extraction Buffer
Tube B	APV-PCR mix (Green cap)
Tube C	Specific APV Activation Buffer (Blue cap)
Tube D	Specific APV 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 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 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.

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.

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 mm² 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 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 APV-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific APV-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.

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

(3) Into a third clean reaction vial add **5µl APV-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific APV 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. **56°C for 30 seconds**

D. **72°C for 30 seconds**

End cycles

E. **72°C for 2 minutes**

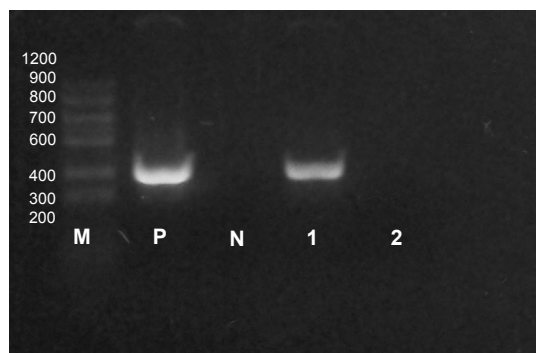
F. **Stop at 8°C**

(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 **440bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **440bp**.

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 APV.

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.
- To avoid false positive interpretation, check vaccination schedules. PCR may be positive 2-6 weeks post vaccination.

XI. References

- Johne R et al. (2006) Characterization of two novel polyomaviruses of birds by using multiply primed rolling-circle amplification of their genomes. *J. Virol.* 80:3523–3531.
- Ogawa H et al. (2005) Duplex shuttle PCR for differential diagnosis of budgerigar fledgling disease and psittacine beak and feather disease. *Microbiol Immunol.* 49(3):227-37.
- Phalen D et al. (1999) Genetic diversity in twenty variants of the avian polyomavirus. *Avian Dis.* 43:207–218.
- Rossi G et al. (2005) Outbreak of avian polyomavirus infection with high mortality in recently captured Crimson's seedcrackers (*Pyrenestes sanguineus*). *J Wildl Dis.* 41(1):236-40.
- Todd D (2004) Avian circovirus diseases: lessons for the study of PMWS. *Vet Microbiol.* 4:98(2):169-74

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.