

# Ready To Use PCR Reagents

## PSITTACID HERPES VIRUS

Cat. No. 60PHV100  
INSTRUCTION MANUAL

### I. Intended Use

**PHV Ready to Use PCR Reagents** are intended for Psittacid Herpes 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 PHV via PCR. No purification is needed! Tubes B, C and D are the components for subsequent use in PCR amplification. Tube B contains **PHV-PCR mix**, Tube C contains **PHV 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 PHV-PCR mix**, **10µl PHV 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 **280bp** band.

### III. Description Of The Disease

The Psittacid Herpesvirus (PHV), an  $\alpha$ -herpesvirus, causes Pacheco's Disease (PD), an acute, generally fatal disease of psittacine birds (parrots). Many parrot species from different regions are susceptible to PD. However, Amazon parrots (*Amazona* spp.) account for the majority of cases, followed by African grey parrots (*Psittacus erithacus*), macaws (*Ara* spp.), cockatoos (*Cacatua* spp.), and conures (*Aratinga* spp. and *Pyrrhura* spp.). Increasing evidence suggests that one or more PHV genotypes may also cause internal papillomatosis of parrots, which manifest in wart-like lesions in oral cavity, upper digestive system and cloacae. This disease is common in both imported and captive-raised parrots. Serotypes: serotype 1 is probably the most common cause of PD, followed by serotype 2 and, less frequently, serotype 3. Serotypes 4 and 5 are represented by only single isolates. Additional genotypes have been reported around the world. Genotypes 1 - 10 are known to cause PD.

### IV. Diagnosis Of The Disease

The use of a PCR that is capable of detecting PHVs in tissues at necropsy and blood in live birds is of value in controlling the impact of these viruses leading to PD. Rapid postmortem diagnosis allows aviculturalists to immediately begin medicating exposed birds and make management changes that would reduce mortality rates. PCR is also valuable adjunct to the pathologist when the histologic lesions are suggestive but not pathogenomic for PD. In live bird, PHV may be found on mucous membranes or in blood, so apparently asymptomatic birds can be identified and isolated from susceptible birds. A single primer set can amplify all 10 variants of the PHV. PCR had been found to be a highly sensitive assay for the detection of PHVs in tissues and culture fluids.

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

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

### 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 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

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### 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<sup>2</sup> 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 mm<sup>2</sup> 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 PHV-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific PHV-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl PHV-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific PHV Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl PHV-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific PHV 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:

- 95°C for 2 minutes**
- 38 cycles of:**
- 94°C for 30 seconds**
  - 50°C for 30 seconds**
  - 72°C for 30 seconds**
- End cycles**
- 72°C for 2 minutes**
  - Stop at 8°C**

- (5) If not used immediately, store PCR products at 4°C until application on Agarose.

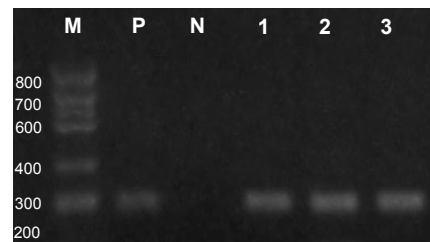
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## IX. Reading And Interpreting The Results

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- 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 **280bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **280bp**.

**Fig. 1 - Visualization of the PCR product.**



Lanes: M Size Marker, P Positive Control, N Negative Control. Lanes 1 - 3 are test samples which are positive for PHV

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## X. Limitations And Troubleshooting

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- 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 PHV vaccination.

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## XI. References

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- Tomaszewski EK et al. (2001) Detection and heterogeneity of herpesviruses causing Pacheco's disease in parrots. J Clin Microbiol. 39(2):533-8.
- Tomaszewski EK et al. (2003) Molecular phylogeny of the psittacid herpesviruses causing Pacheco's disease: correlation of genotype with phenotypic expression. J Virol. 77(20):11260-7.
- Tomaszewski EK et al. (2006) Tissue distribution of psittacid herpesviruses in latently infected parrots, repeated sampling of latently infected parrots and prevalence of latency in parrots submitted for necropsy. J Vet Diagn Invest. 18(6):536-44.

For further information and assistance please contact your local distributor or Biogal Galed Labs. Directly by e-mail: [info@biogal.co.il](mailto:info@biogal.co.il) or by tel: 972-4-9898605 / fax: 972-4-9898690.