



Ready To Use PCR Reagents

CANINE HERPESVIRUS

Cat. No. 60CHV100

INSTRUCTION MANUAL

I. Intended Use

CHV Ready to Use PCR Reagents are intended for Herpesvirus 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 **CHV** via PCR. No purification is needed! Tubes **B**, **C** and **D** are the components for subsequent use in PCR amplification. Tube **B** contains **CHV-PCR mix**, Tube **C** contains **CHV 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 CHV-PCR mix**, **10µl CHV 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 **320bp** band.

III. Description Of The Disease

Canine herpesvirus-1 (CHV-1) was first recognized as an agent responsible for causing a fatal hemorrhagic disease in newborn puppies in 1965. The virus has been isolated in numerous countries and recent studies suggest it to be enzootic in dog population. Apart from clinical papulovesicular genital lesions, CHV-1 can also be involved in reproductive disorders such as embryonic resorption, abortion and stillbirth. The virus is furthermore associated with respiratory (kennel cough syndrome) and ocular disease in immature and adult dogs; latency in sensory ganglia has been also demonstrated. Oronasal and venereal transmission are considered to be the main routes of infection, but fetuses can be infected in uterus.

IV. Diagnosis Of The Disease

The virus is considered to be poorly immunogenic as neutralizing antibodies disappear within a few months after infection. CHV-1 pathology is highly influenced by factors altering immunity (such as age, pregnancy, stress, immunosuppressive therapy and concomitant diseases). CHV polymerase chain reaction (PCR) recently developed to determine the virus infection, has shown promising sensitivity and reliability using blood samples.

V. Contents (Sufficient for 48 tests)

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

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

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

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

(4) Gently mix each reaction vial (do no 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. 60°C for 30 seconds

D. 72°C for 30 seconds

End cycles

E. 72°C for 2 minutes

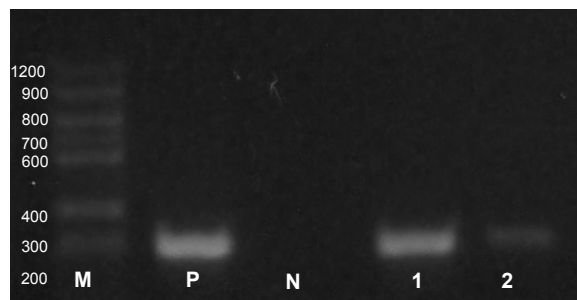
F. Stop at 8°C

(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 **320bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **320bp**.

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 and 2 are positive for CHV.

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

- Burr PD et al. (1996) Detection of canine herpesvirus in a wide range of tissues using the polymerase chain reaction. *Vet Microbiol* 53:227–37.
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- Miyoshi M et al. (1999) Detection of canine herpesvirus dna in the ganglionic neurons and the lymph node lymphocytes of latently infected dogs. *J Vet Med Sci*. 61:375–9.
- Ronsse V et al. (2005) Canine herpesvirus-1 (CHV-1): clinical, serological and virological patterns in breeding colonies. *Theriogenology*. 1;64(1):61-74. Epub 2004 Dec 25.

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.