



Ready To Use PCR Reagents

CANINE DISTEMPER VIRUS

Cat. No. 60CDV100
INSTRUCTION MANUAL

I. Intended Use

CDV Ready to Use PCR Reagents are intended for Canine Distemper Virus amplifications. All reagents are ready to use for a successful amplification of the viral cDNA and obtaining PCR products suitable for loading onto an Agarose gel.

II. General Information

Each package contains **cDNA Diluting Buffer** (Tube **A**) which is intended to dilute the cDNA prior to the PCR amplification. The remaining 3 tubes are the components for subsequent use in PCR amplification. Tube **B** contains **CDV-PCR mix**, Tube **C** contains **CDV Activation Buffer** and Tube **D** contains the **Positive Control**. The Diluting Buffer (Tube **A**) also serves as **Negative Control**. Each PCR set up should include 3 reaction vials; each vial should be added with: **5µl CDV-PCR mix**, **10µl CDV Activation Buffer** and **5µl DNA product of the Diluting 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 **290bp** band.

III. Description Of The Disease

Canine distemper is a contagious, incurable, often fatal, multisystemic viral disease that affects the respiratory, gastrointestinal and central nervous systems. Distemper is caused by the canine distemper virus (CDV) which is part of the Morbillivirus genus (Paramyxoviridae family). Canine distemper occurs worldwide, and once was the leading cause of death in unvaccinated puppies. Widespread vaccination programs have dramatically reduced its incidence. CDV occurs among domestic dogs and many other carnivores, including raccoons, skunks, and foxes. CDV is fairly common in wildlife and in large cats such as lions, leopards, cheetahs and tigers.

IV. Diagnosis Of The Disease

The most efficient and sensitive technique to identify a CDV infection is by amplification of the pathogens genome in blood or cerebrospinal fluid (CSF). Since the genome of all morbilliviruses consists of a single strand of RNA, it must first be copied into cDNA, using reverse transcriptase, in a two steps reaction: transcription / polymerase chain reaction (RT-PCR). RT-PCR has been shown to be useful for the rapid detection of morbillivirus-specific RNA in samples submitted for laboratory diagnosis. A positive amplification of the cDNA means that the virus RNA is present in the tested sample.

V. Contents (Sufficient for 48 tests)

Tube A	Diluting Buffer
Tube B	CDV-PCR mix (Green cap)
Tube C	Specific CDV Activation Buffer (Blue cap)
Tube D	Specific CDV Positive Control (Red cap)
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VI. Essentials Not Included

RNA Extraction kit.
cDNA Random Priming/Synthesis kit.
RNAase free PCR reaction vials.
PCR Thermo-Cycler.
5-10µ, 100µl Pipettes and RNase free filter tips.
Vortex.
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).

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

(1) Extract total RNA from the blood or CSF sample using one of the commercial available **RNA extraction kits** following the vendor's protocol, including purity and quality validation. If not used immediately, store extract at -70°C.

(2) Prepare **cDNA** using **random priming** from one of the commercial cDNA kits according to the vendor's instructions. If not used immediately, store extract at -70°C.

(3) If RNA extraction yielded more than 1 µg of total RNA per 20 µl cDNA preparation, dilute the cDNA in 1:1 V/V with the Diluting buffer (Tube A).

(4) Into a clean reaction vial add: **5µl CDV-PCR mix** (Tube B), **5µl of the Diluted cDNA product** and **10µl of the specific CDV-Activation Buffer** (Tube C). Mark each reaction vial properly to avoid mistakes.

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

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

(7) Gently mix each reaction vial and place in the thermal cyclor 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**

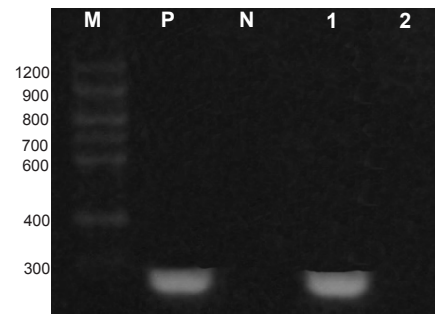
F. **Stop at 8°C**

(8) 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 **290bp**.
- No band should be detected at the Negative Control lane.
- The expected product only band should be at **290bp**.

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 CDV.

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

- Barrett T (1999) Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. *Vet Microbiol.* 1;69(1-2):3-13.
- Demeter Z et al. (2007) Genetic diversity of Hungarian canine distemper virus strains. *Vet Microbiol.* 21;122(3-4):258-69. Epub 2007 Feb 8.
- Kapil S et al. (2008) Canine distemper virus strains circulating among North American dogs. *Clin Vaccine Immunol.* 15(4):707-12. Epub 2008 Feb 6.
- Lan NT et al. (2007) Comparison of molecular and growth properties for two different canine distemper virus clusters, Asia 1 and 2, in Japan. *J Vet Med Sci.* 69(7):739-44.

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.