



# Ready To Use PCR Reagents

## CANINE CORONAVIRUS

Cat. No. 60CCV100  
INSTRUCTION MANUAL

### I. Intended Use

**CCV Ready to Use PCR Reagents** are intended for Canine Coronavirus amplifications. All reagents are ready to use for a successful amplification of the viral cDNA and obtaining PCR products suitable for loading onto 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 **CCV-PCR mix**, Tube **C** contains **CCV 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 CCV-PCR mix**, **10µl CCV 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 **177/270bp** band.

### III. Description Of The Disease

Coronaviruses (family Coronaviridae, order Nidovirales) are large, single-stranded, positive-sense RNA viruses, which are responsible for enteric and/or respiratory diseases in mammals and birds. Several CCV outbreaks have been reported worldwide, showing that CCV is an important enteropathogen of dogs. Serological and virological investigations have demonstrated that CCV is widespread in dog population, mainly in kennels and animal shelters. The incidence of the disease is not known. Yet while present, it is heavily shed in feces of infected dogs. CCV infection is restricted to the alimentary tract, leading to the onset of clinical signs typical of the gastroenteric involvement including loss of appetite, vomiting, fluid diarrhoea, dehydration and, only occasionally, death. Usually, systemic disease is not observed during CCV infection, although the virus has been isolated from several tissues (tonsils, lungs and liver) of experimentally infected pups. Coronavirus infection makes the intestinal cells more susceptible to parvovirus infection. Fatal disease commonly occurs as a consequence of mixed infections with CCV together with canine parvovirus type 2 (CPV-2).

### IV. Diagnosis Of The Disease

The sensitivity as well as specificity of antigen detection by culturing respiratory samples, is low. Molecular detection of the virus using PCR, is far more effective, rapid, sensitive and specific. The virus can be found with onset of the clinical signs (respiratory or enteropathy), in both feces and nasal swabs. Using PCR, the virus can also be tracked in the blood stream, liver, lung and spleen.

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

|        |   |
|--------|---|
| Tube A | Diluting Buffer                           |
| Tube B | CCV-PCR mix (Green cap)                   |
| Tube C | Specific CCV Activation Buffer (Blue cap) |
| Tube D | Specific CCV Positive Control (Red cap)   |
|        | CCV Instruction Manual                    |

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

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

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

(7) Gently mix each reaction vial 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. **53°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.

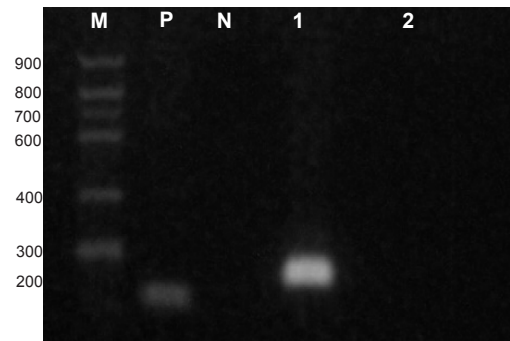
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## IX. Reading And Interpreting The Results

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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 **177bp**.
- No band should be detected at the Negative Control lane.
- The expected product only band should be at **177/270bp**.

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 CCV.

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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 CCV vaccination.
- PCR results can only be interpreted in correlation with clinical symptoms.vaccination.

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## XI. References

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- Binn LN, et al. (1974) Recovery and characterization of a coronavirus from military dogs with diarrhea. Proc. Annu. Meet. U.S. Anim. Health Assoc. 78: 359–366.
- Buonavoglia C et al. (2006) Canine coronavirus highly pathogenic for dogs. Emerg. Infect. Dis. 12: 492–494.
- Lorusso A et al. (2007) Identification and biochemical characterization of a novel protein unique to canine coronavirus tyel. In: Proceedings of the Third European Congress of Virology “EuroVirology 2007”, Nuernberg: 1–5.

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.