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For research use only

Cat. No. IP12071 | **48 Tests**



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Canine coronavirus Detection Kit

Test for the detection of Canine Coronavirus by one-step RT-PCR

User Manual

REV.2.2

7. NOTICE

- This product was designed to detect more than 100 copies of target gene(or gene segment). When the copy number of target present in the test reaction is less than 100, a false-negative(a negative test result when the attribute for which the subject is being tested actually exists in that subject) may occur. Use this product For Research Use Only.
- Do not use any reagent after the expiration date.
- Do not use together with reagents of other products.
- Follow the instructions.
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. iNtRON Biotechnology, Inc. does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

8. TROUBLE SHOOTING

- ① In the case of difficult to interpret results due to non-specific bands.
 - ☞ Reduce amount of template by 1/10 dilution and reacts again.
- ② Preparation of PCR reaction at room temperature may cause the non-specific band.
- ③ All procedure should be carried out on ice.

9. ORDERING INFORMATION

Product	Catalog No.
Canine coronavirus Detection Kit	IP12071
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
SiZer™ 100 DNA Marker	24073

1. DESCRIPTION

Canine coronavirus (CCoV) is a cause of sporadic outbreaks of enteritis in dogs. The infection was first described by Binn in 1971 during an epizootic in Germany, although serological evidence was obtained earlier suggesting that dogs may be naturally infected with a coronavirus related to swine transmissible gastroenteritis virus (TGEV). CCoV is now recognized as an important, but imperfectly understood, pathogen of dogs.

The incubation period is short. Vomiting and diarrhea may be seen by 1 - 3 days post-infection and, when clinical illness occurs, virus spreads rapidly. The virus is highly contagious and often may cause clinical signs in some dogs, with no illness occurring in other contact animals. Feces may be mucoid or watery, sometimes streaked with blood, and it is exceptionally malodorous. Pups become dehydrated, depressed and anorexic, even if fluid therapy is started early. There is generally no fever, although elevated body temperatures have been observed in some cases.

Canine coronavirus Detection Kit is direct detection of canine coronavirus on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the RT-PCR (Reverse Transcription-Polymerase Chain Reaction) method, and take only 2~3 hours for detection. Therefore, it is a very fast accurate, reliable technique.

2. STORAGE

The components of **Canine coronavirus Detection Kit** should be stored at -20 °C, under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

Canine coronavirus RT-PCR Pre-mixture	48 tubes
DNase/RNase-free water (white cap)	1 vial
CCoV positive control (Yellow cap)	2 vial

Component in 20µl reaction

OptiScript™ RT System
 RT-PCR buffer
 dNTPs
 i-StarTaq™ DNA Polymerase
 Chemical stabilizer
 8-MOP (dissolved in DMSO)
 Primers for CCoV

4. SPECIMEN

Performs the test with whole blood, fecal swab or feces. The specimen should be stored at -20 °C prior to use.

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- RNA extraction kit (see 6.1 RNA preparation method)
- Pipettes
- Sterile pipette tip
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cycler
- Electrophoresis kit
- UV transilluminator

6. PROCEDURE

Please read through the entire procedure before starting.

6.1 RNA Preparation

Various manufacturers offer RNA isolation kits. Please carry out the RNA isolation according to the manufacturer’s instructions. The following standard extraction kit is recommended.

Product	Catalog No.	Manufacturer
Viral gene-spin™ Viral DNA/RNA Extraction Kit	17151	iNtRON Biotechnology, Inc.

6.2 Amplification

- ① Prepare appropriate RT-PCR premix tubes and label. And one RT-PCR premix tube for positive control.
- ② Add 2µl of template RNA into the RT-PCR premix tube.
- ③ Add 18µl of DNase/RNase-free water into the RT-PCR premix tube to total volume as 20µl.
- ④ Add 2µl of positive control and 18µl of RNase-free water into a RT-PCR premix tube for monitoring of amplification and easy interpretation.
- ⑤ Dissolve the blue pellet by pipetting.
Note :The pellet is easily dissolved, by letting the mixture stand at R.T. for 1-2minutes after adding water.
- ⑥ (Optional) Add mineral oil. This step is unnecessary when using a thermal cycler that employs a top heating method (general methods).
- ⑦ Perform PCR reaction of samples as the below process using PCR machine.

RT-PCR cycle		Temp.	Time
1 Cycle	Reverse transcription reaction	45℃	30 min.
	Inactivation of reverse transcriptase	94℃	5 min
40 Cycles	Denaturation	94℃	30 sec.
	Annealing	50℃	30 sec.
	Extension	72℃	40 sec.
1 Cycle	Final extension	72℃	5 min.

6.3 Detection of Amplified Products

- ① Prepare 1.5% agarose gel containing RedSafe™ Nucleic Acid Staining Solution. (Cat. No. 21141)
- ② Load 7µl of PCR product and positive control on agarose gel without adding a loading-dye buffer and perform electrophoresis.
- ③ Run electrophoresis by 100V (required about 30~40 minutes).
- ④ Identify the result on ultra-violet (UV) transilluminator.

6.4 Interpretation

- Expected PCR product size : 241 bp

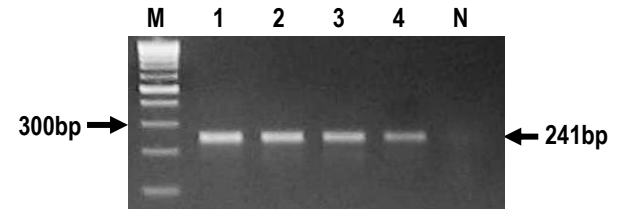


Fig 1. Electrophoresis of PCR product by **Canine coronavirus Detection Kit**
 Lane M : 100bp molecular ladder (iNtRON Biotechnology)
 Lane 1~4 : CCoV positive sample
 Lane N : Negative control

6.5 Elimination of carry-over contamination

- Each PCR/RT-PCR Pre-mixture contains 8-methoxypsoralen (8-MOP) for preventing of carry-over contamination.
- All PCR products should be discarded after UV irradiation (10 min/365nm) for preventing from carry-over contamination.