ISO 9001/14000 certified

For research use only

Cat. No. IP13071 | **24 Tests**

Canine distemper virus Detection Kit

Test for the detection of Canine distemper virus by nested-PCR

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User Manual

REV.2.2

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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 distemper virus Detection Kit	IP13071
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
SiZer ™ 100 DNA Marker	24073

6.5 Interpretation

- Expected CDV 2nd PCR product size : **264 bp**

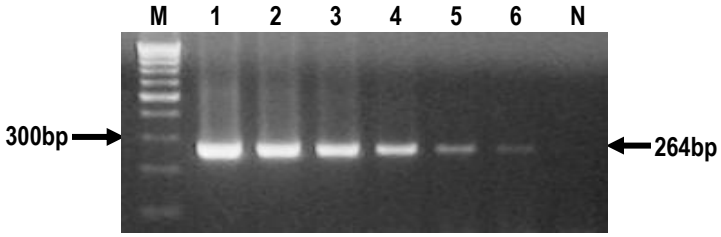

Fig 1. Electrophoresis of PCR product by **Canine distemper virus Detection Kit**
Lane M : 100bp molecular ladder (iNtRON Biotechnology)
Lane 1~6 : CDV positive sample
Lane N : Negative control

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.

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.

1. DESCRIPTION

Canine distemper is a highly infectious, acute or subacute, febrile viral disease of dogs and other carnivores, which occurs world-wide. The disease is caused by canine distemper virus (CDV), a member of the genus *morbillivirus* which belongs to the *paramyxovirus* family.

There is great variation in the duration and severity of clinical disease. Signs may range from no visible signs to severe disease, with or without CNS signs, with approximately 50% mortality. The first pyrexia (3 - 6 DPI) may pass unnoticed, the second peak (several days later and intermittent thereafter) is usually connected with serous (later mucopurulent) nasal and ocular discharge, depression and anorexia. Lymphopenia is always present during the early infection. Gastrointestinal and/or respiratory signs may follow, often enhanced by secondary bacterial infection. However, about 50% of CDV infections in dogs are probably subclinical or very mild. Some dogs develop CNS signs, often, but not always, following systemic disease. Depending on the virus strain, the signs may be more related to acute grey matter or subacute white matter disease.

Canine distemper virus *Detection Kit* is direct detection of canine distemper virus on the basis of a genetic data base, 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 3~4 hours for detection. Therefore, it is a very fast accurate, reliable technique.

2. STORAGE

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

3. CONTENTS

Canine distemper virus 1st RT-PCR Pre-mixture (violet) ····· 24 tubes
Canine distemper virus *2nd* PCR Pre-mixture (yellow)····· 24 tubes
DNase/RNase-free water (white cap) ····· 1 vial
CDV positive control (yellow cap) ····· 2 vial

Component in RT-PCR reaction	Component in 2nd PCR reaction
OptiScript™ RT System	i-StarTaq™ DNA Polymerase
RT-PCR buffer	dNTPs
dNTPs	PCR Reaction buffer
i-StarTaq™ DNA Polymerase	Chemical stabilizer
Chemical stabilizer	Gel loading buffer
8-MOP (dissolved in DMSO)	8-MOP (dissolved in DMSO)
1st Primers for CDV	2nd Primers for CDV

4. SPECIMEN

Performs the test with whole blood, feces, CSF or tissue sample. The specimen should be stored at -20℃ 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 (RT-PCR)

① Prepare appropriate RT-PCR premix tubes(violet) and label. And one RT-PCR premix tube(violet) for positive control.
② Add 2㎕ of template RNA into the RT-PCR premix tube(violet).
③ Add 18㎕ of DNase/RNase-free water into the RT-PCR premix tube(violet) to total volume as 20㎕.
④ Add 2㎕ of positive control and 18㎕ of RNase-free water into a RT-PCR premix tube(violet) 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 RT-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 Amplification (2nd PCR)

① Prepare appropriate 2nd PCR premix tubes(yellow) and label. And one 2nd PCR premix tube(yellow) for positive control.
② Add 1㎕ of RT-PCR product into the 2nd PCR premix tube(yellow).
③ Add 19㎕ of DNase/RNase-free water into the 2nd PCR premix tube(yellow) to total volume as 20㎕.
④ Add 1㎕ of positive control and 19㎕ of RNase-free water into a 2nd PCR premix tube(yellow) 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 Perform PCR reaction of samples as the below process using PCR machine.

2nd PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94℃	5 min.
30 Cycles	Denaturation	94℃	5 sec.
	Annealing	45℃	5 sec.
	Extension	72℃	15 sec.
1 Cycle	Final extension	72℃	5 min.

6.4 Detection of Amplified Products

① Prepare 1.5% agarose gel containing RedSafe™ Nucleic Acid Staining Solution. (Cat. No. 21141)
② Load 7㎕ 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.