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For research use only
Cat. No. IP11074 | 48 **Tests**

Canine parvovirus Detection Kit

Test for the detection of Canine Parvovirus (type-1 and type-2) by one-step PCR

User Manual

REV.2.2

Table of Contents

1. **Description** ··· 1
2. **Storage** ··· 1
3. **Contents** ··· 2
4. **Specimen** ··· 2
5. **Additional required materials** ··· 2
6. **Procedure** ··· 2
   - 6.1 DNA preparation ··· 2
   - 6.2 Amplification ··· 3
   - 6.3 Detection of amplification product ··· 3
   - 6.4 Interpretation ··· 4
   - 6.5 Elimination of carry-over contamination ··· 4
7. **Notice** ··· 4
8. **Trouble shooting** ··· 5
9. **Ordering information** ··· 5

Canine parvovirus Detection Kit

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 parvovirus Detection Kit	IP11074
Viral Gene-spin^{TM} Viral DNA/RNA Extraction kit	17151
SiZer ^{TM} 100 DNA Marker	24073

6.4 Interpretation

- Expected CPV-1 PCR product size : **406 bp**
- Expected CPV-2 PCR product size : **257 bp**

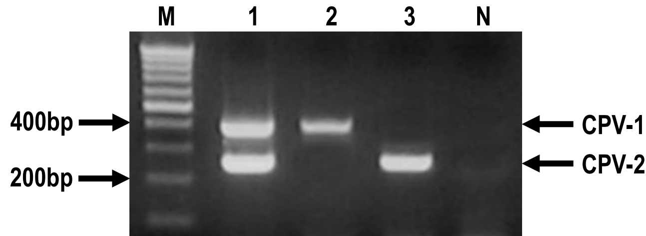

Fig 1. Electrophoresis of PCR product by **Canine parvovirus Detection Kit**
Lane M : 100bp Molecular ladder (iNtRON Biotechnology)
Lane 1 : CPV type-1 and type-2 positive sample
Lane 2 : CPV type-1 positive sample
Lane 3 : CPV type-2 positive sample
Lane N : negative control

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

Two distinct parvoviruses (CPV), are now known to infect dogs - the pathogenic CPV-2, which was recognized as a new disease of dogs and wild canines in 1978, and the “canine minute virus" (CMV, CPV-1) reported by Binn in 1970. MVC, a completely different parvovirus, had not been associated with natural disease until 1992. CMV may cause pneumonia, myocarditis and enteritis in young pups, or transplacental infections in pregnant dams, with embryo resorptions and fetal death. Confirmed infections have been reported in the USA, Sweden, Germany, and, more recently in Italy. Only about 30 cases have been reported.

Canine parvovirus (CPV, CPV-2) and feline panleukopenia virus (FPV) are very closely related and are important pathogens of their respective hosts, the dog and cat. CPV-2 infects dogs and other Canidae such as wolves, coyotes, South American dogs and Asiatic raccoon dogs, but not cats. FPV and the FPV-like viruses infect both large and small cats, as well as mink, raccoons, and possibly foxes, but not dogs. However, the clear separation of a cat virus infecting only cats (FPV) and a dog virus infecting only dogs (CPV-2) is no longer certain as the original dog virus, CPV-2 was transitory, and replaced in nature by so-called "new antigenic types" (CPV-2a and CPV-2b) that infect or replicate in, and are transmitted between, dogs and cats.

Clinical signs of CPV are well known, and only briefly reviewed here since they have been reviewed in several publications. Disease is often asymptomatic in older dogs or in pups that receive a low virus dose since the severity of infection is highly dose related. For example, a pup may acquire infection by CPV in a contaminated kennel, dog show, or veterinary clinic and experience only mild, or no illness. However, virus amplified in the intestine of that pup would be shed in large amounts to littermates or other susceptible dogs in contact. In contrast to the marked panleukopenia seen in cats infected with FPV, a relative lymphopenia, not panleukopenia, is often observed in dogs infected with CPV. Lymphocyte numbers decline, but there is little effect on eosinophil, basophil, monocyte, or red cell numbers.

Canine parvovirus Detection Kit is direct detection of canine parvovirus type-1/type-2 on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the PCR (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 parvovirus Detection Kit*** should be stored at -20℃, under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

Canine parvovirus PCR Pre-mixture ········ 48 tubes
DNase/RNase-free water (white cap) ········ 1 vial
CPV positive control (Yellow cap) ········ 2 vial

Component in 20μℓ reaction
i-StarTaq™ DNA Polymerase dNTPs Reaction buffer Chemical stabilizer Gel loading buffer 8-MOP (dissolved in DMSO) Primers for CPV type-1 and type-2

4. SPECIMEN

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

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- DNA extraction kit (see 6.1 DNA 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 DNA Preparation

Various manufacturers offer DNA isolation kits. Please carry out the DNA 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 PCR premix tubes and label. And one PCR premix tube for positive control.

② Add 2μℓ of template DNA into the PCR premix tube.

③ Add 18μℓ of DNase/RNase-free water into the PCR premix tube to total volume as 20μℓ.

④ Add 2μℓ of positive control and 18μℓ of RNase-free water into a 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.

PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94℃	5 min.
40 Cycles	Denaturation	94℃	30 sec.
	Annealing	52℃	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.