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For research use only

Cat. No. IP12176 | 48 **Tests**



Distribuito in ITALIA da  
**Li StarFish S.r.l.**  
Via Cavour, 35  
20063 Cernusco S/N (MI)  
telefono 02-92150794  
fax 02-92157285  
info@listarfish.it  
www.listarfish.it

## **Feline Coronavirus Detection Kit**

Test for the detection of Feline Coronavirus(FCoV) or Feline Infectious Peritonitis Virus(FIPV) 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.
Feline Coronavirus Detection Kit	IP12176
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
SiZer™ 100 DNA Marker	24073

**1. DESCRIPTION**

Feline infectious peritonitis (FIP) is a serious, nearly always fatal systemic viral disease of wild and domestic cats caused by a coronavirus, a type of virus that afflicts pigs, dogs, and in some forms, humans.

The clinical name for the virus that causes FIP is feline coronavirus. A common, relatively benign form of feline coronavirus is the feline enteric coronavirus (FECV). When FECV mutates into a disease-causing form, it is called FIPV (feline infectious peritonitis virus). If a cat's immune system responds poorly, the mutant FECV may cause the systemic infection called FIP. In wet FIP, fluid accumulates in body cavities-typically in the abdominal cavity, where it causes progressive, but generally painless, swelling; or in the thoracic (chest) cavity, where it causes respiratory distress due to compression of the lungs and fluid backing up into the airways.

In dry FIP, fluid buildup is minimal and symptoms depend on which organs are affected by lesions. About half of all dry cases produce eye inflammation or neurological problems: paralysis, unsteady gait, and seizures. Other lesions may cause kidney or liver failure. Weight loss, pancreatic disease, depression, anemia, and fever are usually present.

**Feline Coronavirus Detection Kit** is direct detection of feline coronavirus or feline infectious peritonitis virus 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 **Feline Coronavirus Detection Kit** should be stored at -20℃, under this condition, the kit is stable until expiration date stated on the label.

**3. CONTENTS**

<b>Feline Coronavirus</b> RT-PCR Pre-mixture .....	48 tubes
DNase/RNase-free water (white cap) .....	1 vial
FCoV 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 FCoV

#### 4. SPECIMEN

Performs the test with whole blood, feces, anaplastic sarcoma of spleen, liver, pancreas or lung . 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°C	30 min.
	Inactivation of reverse transcriptase	94°C	5 min
40 Cycles	Denaturation	94°C	30 sec.
	Annealing	52°C	30 sec.
	Extension	72°C	40 sec.
1 Cycle	Final extension	72°C	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 : 257 bp

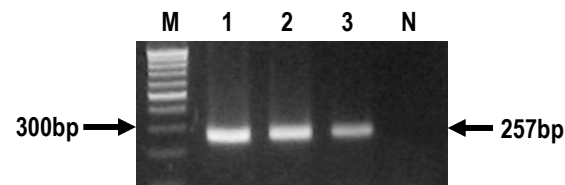


Fig 1. Electrophoresis of PCR product by **Feline Coronavirus Detection Kit**  
 Lane M : 100bp molecular ladder (iNtRON Biotechnology)  
 Lane 1~3 : FCoV positive sample  
 Lane N : Negative control

##### 6.5 Elimination of carry-over contamination

- Each PCR/RT-PCR Pre-mixture contains 8-methoxy psoralen (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.