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For research use only
Cat. No. IP12178 | **48 Tests**

Feline leukemia virus Detection Kit

Test for the detection of Feline Leukemia Virus by one-step RT-PCR

User Manual

REV.2.2

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Feline leukemia virus Detection Kit

■ **NOTE :**

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 leukemia virus Detection Kit	IP12178
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
SiZer ™ 100 DNA Marker	24073

1. DESCRIPTION

Feline leukemia virus (FeLV) infection is responsible for more deaths among cats than any other infectious disease. The virus affects domestic cats and occurs in some wild felines as well. There are three main types of feline leukemia virus: FeLV-A, FeLV-B, and FeLV-C. FeLV-positive cats can be infected with one, two, or all three types: FeLV-A occurs in all FeLV-infected cats and causes severe immunosuppression (weak-ened immune system). FeLV-B occurs in about 50% of all FeLV-infected cats and causes more neoplastic disease (i.e., tumors and other abnormal tissue growths) than cats infected only with FeLV-A. FeLV-C occurs in about 1% of FeLV-infected cats and causes severe anemia.

After the initial infection, the virus replicates in the tonsils and pharyngeal lymph nodes (the pharynx is the muscular tube in the neck). Then it spreads via the bloodstream to other parts of the body, especially the lymph nodes, bone marrow, and intestinal tissue, where it continues to replicate. Viremia, the presence of virus in the blood, usually shows up 2 to 4 weeks after the initial infection.

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

3. CONTENTS

Feline leukemia virus RT-PCR Pre-mixture ····· 48 tubes
DNase/RNase-free water (white cap) ····· 1 vial
FeLV positive control (Yellow cap) ····· 2 vial

Component in 20㎕ reaction
OptiScript™ RT System
RT-PCR buffer
dNTPs
i-StarTaq™ DNA Polymerase
Chemical stabilizer
8-MOP (dissolved in DMSO)
Primers for FeLV

4. SPECIMEN

Performs the test with whole blood, buccal swab, bone marrow or cavity effusion. 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

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

6.4 Interpretation

- Expected PCR product size : **239 bp**

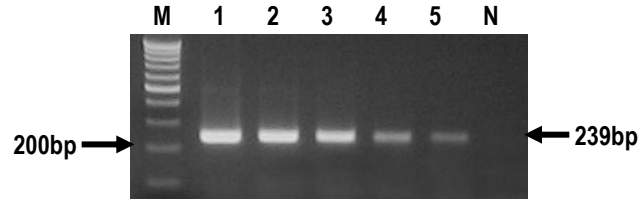

Fig 1. Electrophoresis of PCR product by ***Feline leukemia*** **virus Detection Kit**
Lane M : 100bp molecular ladder (iNtRON Biotechnology)
Lane 1~5 : FeLV positive sample
Lane N : Negative control

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.