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For research use only

Cat. No. IP11177 | 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 immunodeficiency virus Detection Kit

Test for the detection of Feline Immunodeficiency Virus by
one-step 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 immunodeficiency virus Detection Kit	IP11177
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
SiZer™ 100 DNA Marker	24073

1. DESCRIPTION

Feline immunodeficiency virus (FIV) causes an infectious disease in domestic cats and cheetahs similar to human immunodeficiency virus (HIV infection) in humans. It attacks and weakens the body's immune system, making the animal susceptible to infections and diseases that don't affect healthy cats. There is neither a cure nor a vaccine for FIV. Though eventually fatal, an FIV-positive cat can live for many years without any signs of illness.

FIV is transmitted primarily through deep, penetrating bite wounds. A mother cat may transmit the virus to her newborn kittens during gestation, passage through the birth canal, or nursing. FIV can also be transmitted through the transfusion of contaminated blood.

FIV affects only felines. Some of the pathogens (i.e., bacteria, parasites) that cause opportunistic infections in FIV-positive cats may be transmitted from animals to humans and could cause illness in people with compromised immune systems.

Feline immunodeficiency virus Detection Kit is direct detection of feline immunodeficiency virus 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 **Feline immunodeficiency 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 immunodeficiency virus FIV PCR Pre-mixture	48 tubes
DNase/RNase-free water (white cap)	1 vial
FIV positive control (Yellow cap)	2 vial

Component in 20μl reaction

i-StarTaq™ DNA Polymerase
 dNTPs
 PCR Reaction buffer
 Chemical stabilizer
 Gel loading buffer
 8-MOP (dissolved in DMSO)
 Primers for FIV

4. SPECIMEN

Performs the test with whole blood. 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 °C	5 min.
	Denaturation	94 °C	30 sec.
40 Cycles	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 : 553 bp

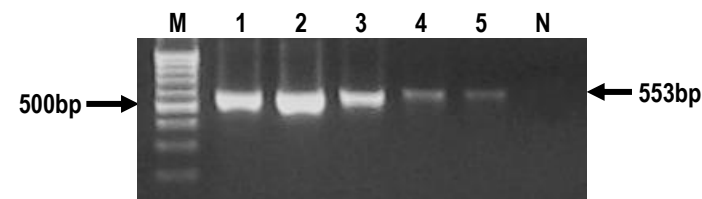


Fig 1. Electrophoresis of PCR product by **Feline immunodeficiency virus Detection Kit**
 Lane M : 100bp Molecular ladder (iNtRON Biotechnology)
 Lane 1~5 : FIV positive sample
 Lane N : Negative control

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.