

1. DESCRIPTION

Feline calicivirus (FCV), member of the family *Caliciviridae*, genus *Vesivirus* is a major cause of acute and chronic upper respiratory diseases in cats. Commonly seen clinical signs include rhinitis, conjunctivitis, stomatitis, gingivitis and glossitis. Less often pneumonia, fever, abortion, cystitis and limping are seen. After resolution of the infection, apparently healthy cats may shed virus for several months or even years. Prevalence rates of feline calicivirus in cats without clinical symptoms vary between 2.5 and 25%. These asymptomatic carrier cats are believed to contribute to the epidemiology of feline calicivirus infection and disease. Recently, virulent feline calicivirus strains associated with a hemorrhagic-like fever and mortality up to 60% in adult cats have been described. Feline calicivirus vaccines have been used widely in the field for the last 20 years. Despite these efforts, feline calicivirus-related illnesses still present a major problem in the feline population especially for cats living in groups.

VetPCR™ FCV Detection Kit is the direct detection of Feline calicivirus 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 4 hours for detection. Therefore, it is a very fast, accurate and reliable technique.

2. STORAGE

The components of VetPCR™ FCV Detection Kit should be stored at -20°C. Under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

| | Kit 48 | Kit 96 | |
|---|-----------|-----------|---------|
| VetPCR™ FCV RT-PCR Pre-mixture | 48 | 96 | tubes |
| VetPCR™ FCV PCR Pre-mixture | 48 | 96 | vial |
| BrigRT-PCR™ solution | 1 | 1 | vial |
| Biotech™ Transcriptase solution | 1 | 1 | vial |
| DNase/RNase-free water | 1 | 1 | vial |
| FCV RT-PCR Positive control | 1 | 1 | vial |
| FCV RT-PCR Positive control Pre-mixture | 4 | 8 | tubes |
| FCV PCR Positive control Pre-mixture | 4 | 8 | tubes |
| Brig™ Molecular Weight marker | 1 | 1 | vial |
| Mineral Oil | 1 | 2 | vial(s) |
| RNA extraction kit (see step 6.1) | 50 | 100 | tests |

4. SPECIMEN

Throat swab or 1 ml tracheal wash, or tissue.

5. ADDITIONAL REQUIRED MATERIALS

- Pipettes, Sterile pipette tip, Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cyler, 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 manufacturers instructions. The following standard RNA Purification kit is recommended.

| Product | Catalog No. | Manufacturer |
|--|-------------|---------------------------------|
| Bioingentech™ Total RNA Purification Kit (50 test) | 230041(50) | Bioingentech Biotechnology Inc. |
| Bioingentech™ Total RNA Purification Kit(100 test) | 230041(100) | Bioingentech Biotechnology Inc. |

6.2 AMPLIFICATION

1.- Prepare appropriate RT-PCR Premix tubes and one RT- PCR Premix tube for Positive control. Label.

2.- Add 5µl of DNase/RNase-free water into the RT-PCR Premix tube to total volume as 8,5µl.

3.- Add 1,5µl of template RNA into the RT-PCR Premix tube to total volume as 10µl.

4.- Add 5µl of DNase/RNase-free water and 1,5µl of RT-PCR Positive control into a RT-PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

5.- Add mineral oil (11µl). This step is necessary, even when using a thermal cyler that employs a top heating method.

6.- Perform RT-PCR reaction (RT-PCR 1) of samples as the below process using a PCR thermal cyler.

7.- Add 0,3µl of BrigRT-PCR™ solution and 0,5µl of Biotech™ Transcriptase solution.

8.- Perform RT-PCR reaction (RT-PCR 2) of samples as the below process, using a PCR thermal cyler.

| RT-PCR cycle | | Temp. | Time |
|--|--------|----------------------|--------------|
| RT-PCR 1 | 1Cycle | Initial Denaturation | 80°C 10 min. |
| | 1Cycle | Stop | 4°C 5 min. |
| Add 0,3µl of BrigRT-PCR™ and 0,5µl of Biotech™ Transcriptase | | | |
| RT-PCR 2 | 1Cycle | Denaturation | 80°C 10 min. |
| | 1Cycle | Annealing | 25°C 10 min. |
| | 1Cycle | Extension | 37°C 50 min. |

9.- Prepare appropriate PCR Premix tubes and one PCR Premix tube for Positive control. Label.

10.- Add 6µl of DNase/RNase-free water into the PCR Premix tube to total volume as 11µl.

11.- Add 2µl of template (cDNA) into the PCR Premix tube to total volume as 13µl.

12.- Add 6µl of DNase/RNase-free water and 2µl of Positive control (Positive control tube from RT-PCR) into a PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

13.- Add mineral oil (11µl). This step is necessary, even when using a thermal cyler that employs a top heating method.

14.- Perform PCR reaction of samples as the below process, using a PCR thermal cyler.

| PCR cycle | | Temp. | Time |
|-----------|----------------------|-------|---------|
| 1 Cycle | Initial Denaturation | 94°C | 2 min. |
| 30 Cycles | Denaturation | 94°C | 30 sec. |
| | Annealing | 57°C | 30 sec. |
| | Extension | 72°C | 30 sec. |
| 1 Cycle | Final extension | 72°C | 5 min. |

6.3 DETECTION OF AMPLIFIED PRODUCTS

- 1.- Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
- 2.- Load 7 μ l of PCR product, 7 μ l of Positive control and 2 μ l of BrigTM Molecular Weight marker 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 : 309 bp

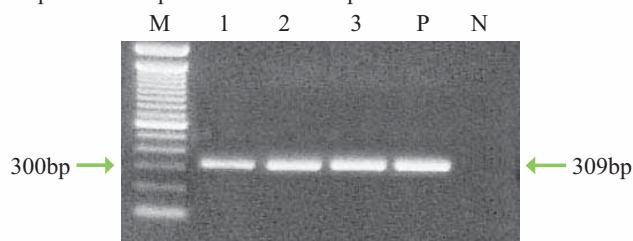


Fig 1. Electrophoresis of PCR product by VetPCRTM FCV Detection Kit
 Lane M : BrigTM Molecular Weight Marker (Bioingentech Ltd.)
 Lane 1~3 : FCV Positive sample
 Lane P : Positive control
 Lane N : Negative control

7. NOTICE

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. Bioingentech 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, heated at 65° C for 5 min. 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. |
|--|---------------|
| VetPCR TM FCV Detection Kit 48 | VET0001FR(48) |
| VetPCR TM FCV Detection Kit 96 | VET0001FR(96) |
| Brig TM Molecular Weight Marker | 24012 |



Bioingentech Ltd.

Salas 350, piso 2, Concepción, Chile
 Telephone (56)-(41)-2790435
 Fax (56)-(41)-2790435
 info@bioingentech.com
 www.bioingentech.com

Product use limitations warranty disclaimer

Bioingentech manufactures product for a number of Intended uses. Please refer to the product label for the Intended use statements for specific product. Bioingentech products contain chemicals which may be harmful if misused. Due care should be exercised with all Bioingentech products to prevent direct human contact. Each Bioingentech product is shipped with documentation stating specifications and other technical information. Bioingentech products are warranted to meet or exceed the stated specifications. Bioingentech's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Bioingentech make no other warranty of any whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTE WITH RESPECT TO BIOINGENTECH PRODUCTS. In no event shall Bioingentech be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Bioingentech products to perform in accordance with the stated specifications.

2004 – 2009 Bioingentech corporation. All rights reserved.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our web site for more information.

All prices and specifications are subject to change without prior notice

Product claims are subject to change. Please contact Bioingentech technical services or access the Bioingentech online catalog for the most up-to-date information on Bioingentech products.