Feline Panleukopenia Virus (FPV) is a feline parvovirus which causes an incurable infectious disease known as feline panleukopenia, feline distemper or feline infectious enteritis. The symptoms of the disease are similar to canine parvo virus. FPV is highly contagious in cats and therefore it is recommended that all cats should be vaccinated as kittens, with booster shots each year. The virus is spread through bodily fluids and contact with feces. Cats may be contagious 2-3 days before symptom development and can spread the disease to other cats up to 6 weeks following recovery. Older cats typically do not develop severe symptoms, however younger, unvaccinated cats can become severely ill. Symptoms of FPV include high fever, vomiting, diarrhea, anorexia, lethargy, seizures and sudden death if severe. Symptoms may develop rapidly and dehydration and shock may result. A cat with FPV should see a veterinarian immediately. There is no cure for FPV, however if a cat survives the first five days of illness it is likely they will survive. Early, reliable diagnosis is therefore important for supportive treatment and control of the spread of the disease.

Principle of the Test
Norgen’s Feline Panleukopenia Virus PCR Detection Kit constitutes a ready-to-use system for the isolation and detection of Feline Panleukopenia Virus using end-point PCR. The kit first allows for the isolation of total DNA, including viral DNA, from the blood samples using spin-column chromatography based on Norgen's proprietary resin. The DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for Feline Panleukopenia Virus detection using the provided FPV Detection Mastermix. The FPV Detection Mastermix contains reagents and enzymes for the specific amplification of a 301 bp region of the viral genome. Norgen’s Feline Panleukopenia Virus PCR Detection Kit contains a second Mastermix, the PCR Control Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the provided PCR control (PCRC) or Isolation Control (IsoC), respectively. This kit is designed to allow for the testing of 24 samples.

Kit Components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Solution</td>
<td>10 mL</td>
</tr>
<tr>
<td>Wash Solution I</td>
<td>9 mL</td>
</tr>
<tr>
<td>Wash Solution II</td>
<td>9 mL</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>6 mL</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>0.6 mL</td>
</tr>
<tr>
<td>Spin Columns</td>
<td>24</td>
</tr>
<tr>
<td>Collection Tubes</td>
<td>24</td>
</tr>
<tr>
<td>Elution tubes (1.7 mL)</td>
<td>24</td>
</tr>
<tr>
<td><strong>FPV 2x PCR Master Mix</strong></td>
<td>0.35 mL</td>
</tr>
<tr>
<td><strong>Control 2x PCR Master Mix</strong></td>
<td>0.35 mL</td>
</tr>
<tr>
<td><strong>Isolation Control (IsoC)</strong></td>
<td>0.3 mL</td>
</tr>
<tr>
<td><strong>FPV Positive Control (PosC)</strong></td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Nuclease Free-Water</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>Norgen's DNA Marker</td>
<td>0.1 mL</td>
</tr>
<tr>
<td><strong>Product Insert</strong></td>
<td>1</td>
</tr>
</tbody>
</table>

*IsoC = Isolation Control; PosC= Positive Control
*The isolation control is a cloned PCR product
*The positive control is a fragment of FPV cloned in a plasmid
Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- Micropipettors
- 2 mL microcentrifuge tubes
- 96 - 100% ethanol
- 55°C waterbath or incubator

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers. The kit contains a ready-to-use Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

The FPV 2x PCR Master Mix, Control 2x PCR Master Mix, FPV Positive Control (PosC) and the Isolation Control (IsoC) should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

General Precautions
The user should exercise the following precautions when using the kit:
- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control
In accordance with Norgen’s ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen’s Feline Panleukopenia Virus PCR Detection Kit, including the FPV 2x PCR Master Mix, Control 2x PCR Master Mix, Isolation Control and FPV Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen’s Feline Panleukopenia Virus PCR Detection Kit is designed for research purposes only.

Product Warranty and Satisfaction Guarantee
NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Precautions
The Lysis Solution and Wash Solution I contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with blood.

Safety Information
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.
Important Notes Prior to Beginning Protocol:

- Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.
- A variable speed microcentrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- For best results, the use of whole blood collected into tubes containing an anticoagulant is highly recommended.
- Both fresh and frozen anticoagulated blood may be used with this procedure. Ensure that frozen blood is thawed at room temperature prior to starting the protocol.
- This kit is also compatible with samples collected using Norgen’s Blood DNA Preservation Buffer (3X) (Cat #29111). Please follow the instructions provided with that product for specimen collection and preservation.
- Prepare a working concentration of Wash Solution I by adding 12 mL of 96-100% Ethanol (provided by user) to the supplied bottle containing concentrated Wash Solution I. This will give a final volume of 21 mL. The label on the bottle has a box that can be checked to indicate that Isopropanol has been added.
- Prepare a working concentration of Wash Solution II by adding 21 mL of 96 – 100 % ethanol (provided by the user) to the supplied bottle containing concentrated Wash Solution II. This will give a final volume of 30 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Always vortex the Proteinase K before use.
- Isolation Control (IsoC)
  - An Isolation Control (IsoC) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the Isolation Control (IsoC) to the lysate during the isolation procedure.
  - The Isolation Control (IsoC) must not be added to the sample material directly.
  - Do not freeze and thaw the Isolation Control (IsoC) more than 2 times.
  - The Isolation Control (IsoC) must be kept on ice at all times during the isolation procedure.
- The PCR components of the Feline Panleukopenia Virus PCR Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
- It is important to work quickly during this procedure.

A. Feline Panleukopenia Virus PCR Assay Preparation

a. Lysate Preparation
1. Add 20 µL of Proteinase K to a microcentrifuge tube.
2. Transfer 200 µL of blood sample to the tube containing Proteinase K.
3. Add 300 µL of Lysis Solution to the blood and mix well by gentle vortexing for 10 seconds.
4. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
5. Incubate at 55°C for 10 minutes.
6. (Optional): If any debris is present in the sample, centrifuge for 2 minutes at 14,000 RPM to precipitate. Transfer the clean supernatant to a microcentrifuge tube prior to Step 7.
7. Add 250 µL of 96-100% Ethanol to the sample and mix well by vortexing for 10 seconds.
8. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

b. Sample Binding to Column
1. Add 10 µL of the Isolation Control (IsoC) to the lysate mixture.
2. Assemble a spin column with a provided collection tube. Apply the lysate to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough. Reassemble the column and the collection tube.

**Note:** Ensure that all of the lysate has passed through into the collection tube. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

c. **Column Wash**
1. Apply 500 µL of **Wash Solution I** (ensure ethanol was added) to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

**Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

2. Apply 500 µL of **Wash Solution II** (ensure ethanol was added) to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

3. Wash column another time by adding 500 µL of **Wash Solution II** and centrifuging for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

4. Spin the column for 2 minutes in order to thoroughly dry the column at 14,000 x g (~14,000 RPM). Discard the collection tube.

d. **DNA Elution**
1. Place the column into a provided 1.7 mL elution tube.
2. Add 200 µL of **Elution Buffer** to the column.
3. Incubate at room temperature for 1 minute.
4. Centrifuge for 1 minute at 6,000 x g (~8,000 RPM)

**B. Feline Panleukopenia Virus PCR Assay Preparation**

**Notes:**
- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of FPV 2X PCR Master Mix and Control 2X PCR Master Mix provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For each sample, one PCR reaction using the FPV 2X PCR Master Mix and one PCR reaction using Control 2X PCR Master Mix should be set up in order to have a proper interpretation of the results.
- For every PCR run, one reaction containing FPV Positive Control and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of Parvo Limit of Detection.

1. Prepare the PCR reaction for sample detection (Set #1, using **FPV 2X PCR Master Mix**) and the PCR reaction for control detection (Set #2, using **Control 2X PCR Master Mix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one Chlamydia detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.
Table 1. PCR Assay Preparation

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPV 2X PCR Master Mix Or Control 2X PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>Sample DNA</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>7.5 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>

2. For each PCR set, prepare one positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPV 2X PCR Master Mix Or Control 2X PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>FPV Positive Control (PosC)</td>
<td>10 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>

3. For each PCR set, prepare one no template control PCR as shown in Table 3 below:

Table 3. PCR Negative Control Preparation

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPV 2X PCR Master Mix Or Control 2X PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>10 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>

C. Feline Panleukopenia Virus PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 4 below.
2. Run one step PCR.
Table 4. Feline Panleukopenia Virus Assay Program

<table>
<thead>
<tr>
<th>PCR Cycle</th>
<th>Step</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cycle 1</strong></td>
<td>Step 1</td>
<td>95°C</td>
<td>3 min</td>
</tr>
<tr>
<td><strong>Cycle 2 (35x)</strong></td>
<td>Step 1</td>
<td>94°C</td>
<td>15 sec</td>
</tr>
<tr>
<td></td>
<td>Step 2</td>
<td>60°C</td>
<td>15 sec</td>
</tr>
<tr>
<td></td>
<td>Step 3</td>
<td>72°C</td>
<td>30 sec</td>
</tr>
<tr>
<td><strong>Cycle 3</strong></td>
<td>Step 1</td>
<td>72°C</td>
<td>5 min</td>
</tr>
<tr>
<td><strong>Cycle 4</strong></td>
<td>Step 1</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

D. Feline Panleukopenia Virus PCR Assay Results Interpretation

1. For the analysis of the PCR data, the entire 15-20 µL PCR Reaction should be loaded on a 1X TAE 1.7% Agarose DNA gel along with 10 µL of Norgen’s DNA Marker (provided).
2. The PCR products should be resolved on the 1X TAE 1.5% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

![Figure 1: A representative 1X TAE 1.5% agarose gel showing the amplification of Feline Panleukopenia Virus at different concentrations (Target). The size of the FPV target amplicon corresponds to 301 bp as represented by the provided DNA Marker (M). NTC = Negative Control.](image-url)
Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of Isolation Control and PCR Control under different conditions using the 2X PCR Control Mastermix. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the DNA isolation as well as the PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the PCR was successful, the isolation failed to recover even the spiked-in Isolation control. NTC = Negative Control.

Table 5. Interpretation of PCR Assay Results

<table>
<thead>
<tr>
<th>Input Type</th>
<th>Target reaction</th>
<th>Control Reaction</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>FPV Band (301 bp)</td>
<td>IsoC Band (499 bp)</td>
<td>PCRC Band (150 bp)</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
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</tr>
<tr>
<td>Sample</td>
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<tr>
<td>Sample</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.
E. Feline Panleukopenia Virus PCR Assay Specificity and Sensitivity

- The specificity of Norgen’s Feline Panleukopenia Virus PCR Detection Kit is first and foremost ensured by the selection of the Feline Panleukopenia Virus specific primers, as well as the selection of stringent reaction conditions. The Feline Panleukopenia Virus specific primers were checked for possible homologies to GenBank published sequences by sequence comparison analysis and published strains.

F. Linear Range

- The linear range of Norgen’s Feline Panleukopenia Virus PCR Detection Kit was determined by analysing a dilution series of a Feline Panleukopenia Virus quantification standards ranging from 100 ag to 1 pg.
- Each dilution has been tested in replicates (n = 4) using Norgen’s Feline Panleukopenia Virus PCR Detection Kit on a 1X TAE 1.7% agarose gel.
- The linear range of Norgen’s Feline Panleukopenia Virus PCR Detection Kit has been determined to cover concentrations from 100 ag to 1 ng
- Under the conditions of the Norgen’s Feline Panleukopenia Virus DNA Isolation procedure, Norgen’s Feline Panleukopenia Virus PCR Detection Kit covers a linear range from 100 copies to $1 \times 10^6$ copies.

Frequently Asked Questions

1. How many samples should be included per PCR run?
   - Norgen’s Feline Panleukopenia Virus PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

2. How can I interpret my results if neither the FPV PCR control nor the FPV Isolation Control (IsoC) amplifies?
   - If neither the FPV PCR control nor the FPV Isolation Control (IsoC) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, whereas if the Positive control did not amplify, therefore the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the FPV PCR control showed amplification but neither the FPV target nor the FPV Isolation control amplified for a sample?
   - This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the FPV Isolation Control (IsoC) was amplified in a sample?
   - The sample tested can be considered as Feline Panleukopenia Virus negative.

5. How should it be interpreted if the FPV PCR control and the FPV target showed amplification in a sample?
   - The sample tested can be considered positive. It could happen when too much template was added to the reaction.

6. How should it be interpreted if only the FPV target and the FPV PCR control were amplified in a sample?
   - The sample tested can be considered as Feline Panleukopenia Virus positive.

7. How should it be interpreted if only the FPV target was amplified in a sample?
   - It is recommended that the isolation is repeated.
8. How should it be interpreted if only the FPV PCR control and the FPV Isolation control showed amplification in a sample?
   • The sample tested can be considered negative

9. What if I forgot to do a dry spin after my third wash?
   • Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

10. What if I forgot to add the Isolation Control (IsoC) during the isolation?
    • It is recommended that the isolation is repeated.

11. What if I forgot to run the Control PCR for the sample and I only ran the Detection PCR and I obtained a positive result?
    • The result can be considered positive. However, any negative result must be verified by running the associated control PCR to ensure that it is a true negative and not a false negative due to problems with the RNA isolation or the PCR reactions.

<table>
<thead>
<tr>
<th>Related Products</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Genomic DNA Isolation Kit</td>
<td>46300</td>
</tr>
<tr>
<td>Blood DNA Preservation Buffer (3X)</td>
<td>29111</td>
</tr>
<tr>
<td><em>Dirofilaria immitis</em> PCR Detection Kit</td>
<td>44500</td>
</tr>
<tr>
<td><em>Leptospira interrogans</em> PCR Detection Kit</td>
<td>44600</td>
</tr>
</tbody>
</table>

**Technical Assistance**

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Feline Panleukopenia Virus PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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