Feline Herpes Virus (FHV) is a common causal agent of upper respiratory disease and eye inflammation in cats. The virus is an α-herpesvirus which is composed of double-stranded DNA. There is only one serotype of the virus. Most cats will be exposed to the virus during their lifetime, and symptoms are typically observed as mild to severe upper respiratory disease. Nasal and ocular discharge is also common. Additional symptoms include conjunctivitis, sneezing, anterior uveitis, stomatitis and may also include salivation and coughing. In severe cases, pneumonia may develop. Herpetic ulcerative dermatitis may also occur which can be observed as chronic, non-healing skin ulcers of the face and nose. Disease is usually self-limiting, although some cats may develop a chronic infection. Kittens are typically more severely affected by the disease. Virus is shed in the oral, conjunctival and nasal secretions of infected cats, with transmission mainly by direct cat-to-cat contact. Infected cats will shed virus for approximately 1 to 3 weeks. Most cats will become lifelong, latently infected carriers, with a proportion of these cats having a recurrence of symptoms and viral shedding following periods of stress or corticosteroid treatment. As the virus is widespread, it is important to have a good molecular diagnosis of the virus in order to distinguish it from other feline virus that causes similar symptoms.

**Principle of the Test**
Norgen’s Feline Herpes Virus PCR Detection Kit constitutes a ready-to-use system for the isolation and detection of FHV using end-point PCR. The kit first allows for the isolation of total DNA, including viral DNA, from the plasma/serum or swab samples using spin-column chromatography based on Norgen’s proprietary resin. The FHV DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for FHV detection using the provided FHV Detection Master Mix. The FHV Detection Master Mix contains reagents and enzymes for the specific amplification of a 338 bp region of the viral genome. Norgen’s FHV PCR Detection Kit contains a second Master Mix, the Control 2x PCR 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>30 mL</td>
</tr>
<tr>
<td>Binding Solution 1</td>
<td>6 mL</td>
</tr>
<tr>
<td>Binding Solution 2</td>
<td>6 mL</td>
</tr>
<tr>
<td>Wash Solution</td>
<td>22 mL</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>3 mL</td>
</tr>
<tr>
<td>Mini Filter 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>FHV 2x PCR Master Mix</strong></td>
<td><strong>0.35 mL</strong></td>
</tr>
<tr>
<td><strong>Control 2x PCR Master Mix</strong></td>
<td><strong>0.35 mL</strong></td>
</tr>
<tr>
<td><strong>Isolation Control (IsoC)</strong></td>
<td><strong>0.3 mL</strong></td>
</tr>
<tr>
<td><strong>FHV Positive Control (PosC)</strong></td>
<td><strong>0.1 mL</strong></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>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

*The isolation control is a cloned PCR product*
*The positive control is a cloned FHV product*
Customer-Supplied Reagents and Equipment
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- 96–100% ethanol
- 60°C incubator

Storage Conditions and Product Stability
All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

The FHV 2x PCR Master Mix, Control 2x PCR Master Mix, the Isolation Control (IsoC) and the FHV Positive Control (PosC) 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 while 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 FHV 2x PCR Master Mix, Control 2x PCR Master Mix, the Isolation Control (IsoC) and the FHV Positive Control (PosC) are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen’s Feline Herpes Virus PCR Detection Kit is designed for research purposes only. It is not intended for human or diagnostic use.

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.

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.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The Lysis Solution, Binding Solution 1 and Binding Solution 2 contain guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.
If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Plasma or Serum 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 plasma or serum.

INSTRUCTIONS FOR USE

Important Notes Prior to Beginning Protocol:

- 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.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
- All centrifugation steps are performed at room temperature.
- A variable speed centrifuge 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.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of the Wash Solution by adding 50 mL of 96-100% ethanol (provided by the user) to the supplied bottles containing the concentrated Wash Solution. This will give a final volume of 72 mL. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to DNA degradation.
- Binding Solution 1 contains resin and must be mixed well before every pipetting.
- 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 FHV Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
- Acceptable specimen types include plasma or serum or nasal/throat swabs or viral culture.
- If using swabs, use only sterile Dacron, nylon or rayon swabs with plastic shafts. Note: Do not use calcium alginate swabs as they may contain substances that are inhibitory to PCR.
- It is recommended that no more than 400 µL of plasma/serum or media be used in order to prevent clogging of the column.
- This kit is suitable for the isolation of DNA from fresh or frozen serum or plasma prepared from blood collected on either Heparin, EDTA or citrate.
- This kit is also compatible with samples collected using Norgen's Sample Collection Kit For Upper Respiratory Tract Infectious Agents (Cat #29100). Please follow the instructions provided with that kit for specimen collection and preservation.
- It is important to work quickly during this procedure.

A. Isolation of DNA

1. In a 2 mL tube add 400 µL Plasma-Serum or viral media sample or swab collected in Norgen’s Sample Collection Kit For Upper Respiratory Tract Infectious Agents.
2. To each 400 µL sample add 1.2 mL of Lysis Solution. Mix well by vortexing for 15 seconds.
3. Incubate the mixture from Step 2 for 10 minutes at 60°C.
4. After incubation add 200 μL of Binding Solution 1 and mix well by vortexing for 15 seconds. 
   *(Note: Binding Solution 1 contains resin and must be mixed well before every pipeting)*
5. Centrifuge the mixture from Step 4 for 1 minute at 2,000 RPM, then carefully decant the 
   supernatant in order to ensure that the slurry pellet is not dislodged.
6. To the slurry pellet from Step 5 add 180 μL from Binding Solution 2 followed by the addition 
   of 420 μL of 96-100% Ethanol (provided by the user) and 10 μL Isolation Control (IsoC). 
   Mix well by vortexing for 15 seconds.
7. Centrifuge the mixture from Step 6 for 1 minute at 2,000 RPM, then carefully decant the 
   supernatant in order to ensure that the slurry pellet is not dislodged.
8. To the slurry pellet from Step 7 add 1 mL Wash Solution, mix well by vortexing for 15 
   seconds and then centrifuge for 1 minute at 2,000 RPM. Carefully decant the supernatant in 
   order to ensure that the slurry pellet is not dislodged.
9. Repeat Step 8 to wash the slurry pellet for a second time.
10. To the slurry pellet from Step 9 add 500 μL Wash Solution, and mix well by vortexing for 15 
    seconds.
11. Transfer the entire mixture from Step 10 into a Mini Filter Spin column. Centrifuge for 1 
    minute at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its 
    collection tube.
12. Spin the column, empty, for 2 minutes at 14,000 RPM. Discard the collection tube;
13. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 μL of Elution Buffer to the 
    column and centrifuge for 2 minutes at 200 x g (~2,000 RPM), followed by 2 minutes at 
    10,000 x g (~14,000 RPM).

B. FHV 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 FHV 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 FHV 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 FHV Positive Control (PosC) 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 FHV Limit of 
  Detection.

1. Prepare the PCR for sample detection (Set #1, using FHV 2x PCR Master Mix) and control 
   detection (Set #2, using 2X PCR Control 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 FHV 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>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHV 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 run, prepare **one** positive control PCR as shown in Table 2 below:

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per RT- PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHV 2x PCR Master Mix or Control 2x PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>FHV Positive Control (PosC)</td>
<td>10 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td>20 µL</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHV 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>20 µL</td>
</tr>
</tbody>
</table>

Therefore, at a minimum, each PCR run will contain 6 separate PCR reactions.

**C. PCR Assay Programming**

1. Program the thermocycler according to the program shown in Table 4 below.
2. Run PCR.

<table>
<thead>
<tr>
<th>One Step 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>5 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>30 sec</td>
</tr>
<tr>
<td></td>
<td>Step 3</td>
<td>72°C</td>
<td>45 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>$\infty$</td>
</tr>
</tbody>
</table>
D. FHV 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). Prepare enough agarose gel for running one set of PCR of FHV detection and one set of PCR for controls detection.

2. The PCR products should be resolved on the 1X TAE 1.7% 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](image1.png)

**Figure 1:** A representative 1X TAE 1.7% agarose gel showing the amplification of FHV under different concentration (FHV Target) using the FHV 2x PCR Master Mix. The size of the FHV target amplicon corresponds to 338 bp as represented by the provided DNA Marker (M). NC = Negative Control.

![Figure 2](image2.png)

**Figure 2:** A representative 1X TAE 1.7% agarose gel showing the amplification of Isolation Control and PCR Control under different conditions using the Control 2x PCR Master Mix. 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. NC = 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></td>
<td>FHV Target Band (338 bp)</td>
<td><em>IsoC Band (499 bp)</em></td>
<td><em>PCRC Band (171 bp)</em></td>
</tr>
<tr>
<td>Positive Control</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
<td></td>
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<td>Sample</td>
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<tr>
<td>Sample</td>
<td>X</td>
<td>X</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. FHV PCR Assay Specificity and Sensitivity
- The specificity of Norgen’s FHV PCR Detection Kit is first and foremost ensured by the selection of the FHV specific primers, as well as the selection of stringent reaction conditions. The FHV specific primers were checked for possible homologies to all GenBank published sequences by sequence comparison analysis and published FHV strains.

F. Linear Range
- The linear range of Norgen’s FHV PCR Detection Kit was determined by analysing a dilution series of a FHV quantification standard ranging from 100 ag to 1 pg.
- Each dilution has been tested in replicates (n = 4) using Norgen’s FHV PCR Detection Kit on a 1X TAE 1.7% agarose gel.
- The linear range of Norgen’s FHV PCR Detection Kit has been determined to cover concentrations from 100 ag to 1 ng.
- Under the conditions of the Norgen’s FHV DNA Isolation procedure, Norgen’s FHV 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 FHV 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 PCR control nor the Isolation Control (IsoC) amplifies?**
   - If neither the PCR control nor the 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, the problem has occurred during the setup of the PCR assay reaction.

3. **How should it be interpreted if only the PCR control showed amplification but neither the FHV target nor the 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 Isolation Control (IsoC) was amplified in a sample?**
   - The sample tested can be considered as FHV negative.

5. **How should it be interpreted if the PCR control and the FHV target showed amplification in a sample?**
   - The sample tested can be considered positive.

6. **How should it be interpreted if only the FHV target was amplified in a sample?**
   - The sample tested can be considered positive.

7. **How should it be interpreted if only the PCR control and the Isolation control showed amplification in a sample?**
   - The sample tested can be considered negative.

8. **What if I forgot to do a dry spin after my last 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.

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

10. **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 DNA isolation or the PCR reactions.
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 Herpes 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.

### Related Products

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Collection Kit For Upper Respiratory Tract Infectious Agents</td>
<td>29100</td>
</tr>
<tr>
<td>Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format)</td>
<td>50600</td>
</tr>
<tr>
<td>Plasma/Serum Circulating Nucleic Acid Purification Mini Kit (Slurry Format)</td>
<td>53300</td>
</tr>
<tr>
<td>Plasma-Serum HSV-2 PCR Detection Kit</td>
<td>32500</td>
</tr>
<tr>
<td>Plasma-Serum HSV-1&amp;2 PCR Detection Kit</td>
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<tr>
<td>Feline Immunodeficiency Virus RT-PCR Detection Kit</td>
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<td>Feline Calicivirus RT-PCR Detection Kit</td>
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<tr>
<td>Feline Leukemia Virus RT-PCR Detection Kit</td>
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<tr>
<td>Feline infectious peritonitis RT-PCR Detection Kit</td>
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