

**Pneumocystis jirovecii PCR Kit**

**Product # 42820**

### Background Information

*Pneumocystis jirovecii* is a yeast-like fungus that causes *Pneumocystis jirovecii* Pneumonia (originally known as *Pneumocystis carinii* Pneumonia or PCP). PCP is the most common opportunistic infection in patients with HIV/AIDS. Species of *Pneumocystis* are commonly found in the lungs of healthy individuals. In fact, most children are believed to be exposed by age 3 or 4 years. Studies have suggested that *P. jirovecii* is communicable, possibly via airborne transmission. Disease usually develops in patients whose cellular immunity and humoral immunity are defective. Even with the widespread use of highly active antiretroviral therapy (HAART), there is still a high prevalence of PCP in HIV patients.

### Product Description

Norgen’s *Pneumocystis jirovecii* PCR Kit is a research use-only kit, based on the use of end-point PCR technology, for the detection of *Pneumocystis jirovecii* specific DNA. The kit includes Master Mix and primers for the specific amplification of a 238 bp region of the *Pneumocystis jirovecii* genome. In addition, the kit contains a positive and a negative control to confirm the integrity of the kit reagents.

The detection of *Pneumocystis jirovecii* specific DNA is based on end-point PCR technology, utilizing polymerase chain reaction (PCR) for the amplification of specific *Pneumocystis jirovecii* DNA sequences. For analysis of the PCR data, the PCR reaction is loaded on an agarose DNA gel along with the provided DNA ladder for qualitative analysis.

Norgen’s *Pneumocystis jirovecii* PCR Kit was developed and validated to be used with the following PCR instruments:

- Qiagen Rotor-Gene Q
- BioRad iCycler

### Kit Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Product # 42820 (48 preps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X PCR Master Mix</td>
<td>2 x 350 µL</td>
</tr>
<tr>
<td><em>P. jirovecii</em> Primer Set Mix</td>
<td>150 µL</td>
</tr>
<tr>
<td><em>P. jirovecii</em> Positive Control</td>
<td>100 µL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>DNA Ladder</td>
<td>200 µL</td>
</tr>
<tr>
<td>DNA Ladder</td>
<td>1</td>
</tr>
</tbody>
</table>

### Storage Conditions and Product Stability

- The *Pneumocystis jirovecii* PCR Kit is shipped on dry ice. The components of the kit should be frozen upon arrival. If one or more of the components is not frozen when the kit is received, or if any of the components have been compromised during shipment, please contact Norgen Biotek for assistance.
- All kit components should be stored at -20°C upon arrival
- All kit components should be stored at -20°C for up to 1 year without showing any reduction in performance.
- Repeated thawing and freezing (> 2 x) of the Master Mix and Positive Control should be avoided, as this may affect the performance of the assay. If the reagents are to be used only intermittently, they should be frozen in aliquots.
Customer-Supplied Reagents and Equipment

- Appropriate End-point PCR Instrument
- DNA Purification Kit
  - The kit is compatible with all DNA purification kits that yield high quality, inhibitor-free DNA
  - **Recommended Purification Kit**: Norgen Biotek’s purification kits for DNA isolation, including:
    - Blood Genomic DNA Isolation Mini Kit - Cat# 46300
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- Agarose gel electrophoresis apparatus
- UV transilluminator with suitable gel documentation system

Quality Control
In accordance with Norgen’s ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen’s *Pneumocystis jirovecii* PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- 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.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the *Pneumocystis jirovecii* genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.
• Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

Instructions for Use

A. Sample Preparation

Purified DNA is the starting material for Norgen’s *Pneumocystis jirovecii* PCR Kit. The quality of the DNA template will have a major impact on the performance of the kit. The user must ensure that the method used for DNA purification is compatible with end-point PCR technology. We recommend the use of Norgen’s purification kits for DNA isolation, including *Norgen’s Blood Genomic DNA Isolation Mini Kit* (Cat# 46300).

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 10 minutes at 14,000 x g (~14,000 RPM), using a new collection tube, is highly recommended prior to the elution of the DNA. This will help to prevent the carry-over of any ethanol into the purified DNA, as ethanol is known to be a strong inhibitor of PCR. Ensure that any traces of ethanol from the sample preparation steps are eliminated prior to the elution of the DNA.

B. PCR Assay Preparation

Notes:
• Before use, suitable amounts of all PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
• Work quickly on ice.
• The amount of 2X PCR Master Mix provided is enough for up to 64 PCR reactions (48 sample PCR, 8 positive control PCR and 8 no template control PCR).
• For every PCR run, one reaction containing *Pneumocystis jirovecii* 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 of sample DNA than recommended may affect the sensitivity of the *Pneumocystis jirovecii* Limit of Detection.
• To avoid any contamination while preparing the PCR assay, follow the order outlined in Tables 1, 2 and 3 below to prepare the Negative Control, Detection Assay and Positive Control:
  1. Prepare the PCR Negative Control (Table 1)
  2. Prepare the PCR *Pneumocystis jirovecii* Assay (Table 2)
  3. Prepare the PCR Positive Control (Table 3)
• To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (ie: 1) Nuclease-free water; 2) Master Mix; 3) Primer Set; and 4) the Sample DNA or Positive Control).

1. For each PCR set, prepare one no template control PCR as shown in Table 1 below:

<table>
<thead>
<tr>
<th>Table 1. PCR Negative Control Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCR Components</strong></td>
</tr>
<tr>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
</tr>
<tr>
<td>2X PCR Master Mix</td>
</tr>
<tr>
<td><em>P. jirovecii</em> Primer Set Mix</td>
</tr>
<tr>
<td>Total Volume</td>
</tr>
</tbody>
</table>
2. Prepare the PCR reaction for sample detection as shown in Table 2 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. 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>Nuclease-Free Water</td>
<td>5.5 μL</td>
</tr>
<tr>
<td>2X PCR Master Mix</td>
<td>10 μL</td>
</tr>
<tr>
<td>P. jirovecii Primer Set Mix</td>
<td>2 μL</td>
</tr>
<tr>
<td>Sample DNA</td>
<td>2.5 μL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>20 μL</td>
</tr>
</tbody>
</table>

3. For each PCR set, prepare one positive 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>Nuclease-Free Water</td>
<td>3 μL</td>
</tr>
<tr>
<td>2X PCR Master Mix</td>
<td>10 μL</td>
</tr>
<tr>
<td>P. jirovecii Primer Set Mix</td>
<td>2 μL</td>
</tr>
<tr>
<td>P. jirovecii Positive Control (PosC)</td>
<td>5 μL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>20 μL</td>
</tr>
</tbody>
</table>

C. *Pneumocystis jirovecii* PCR Assay Programming

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

<table>
<thead>
<tr>
<th>PCR Cycle</th>
<th>Step</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>Step 1</td>
<td>95°C</td>
<td>3 min</td>
</tr>
<tr>
<td>Cycle 2 (40x)</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>Cycle 3</td>
<td>Step 1</td>
<td>72°C</td>
<td>5 min</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>Step 1</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>
D. *Pneumocystis jirovecii* PCR Assay Interpretation

- For the analysis of the PCR data, the entire 20 µL PCR reaction should be loaded on a 1X TAE 2% Agarose DNA gel along with 10 µL of Norgen's DNA Ladder (provided).
- The PCR products should be resolved on the 1X TAE, 2% Agarose gel at 150V for 30 minutes (Gel running time will vary depending on an electrophoresis apparatus).

Valid Test Run

- **Positive Sample**: A sample is determined to be positive only when:
  - Sample lanes show the 238 bp band corresponding to the *Pneumocystis jirovecii* target amplicon
  - Positive Control shows the 238 bp band
  - Negative Control shows no bands

- **Negative Sample**: A sample is determined to be negative only when:
  - Sample lanes contain no bands
  - Positive Control shows the 238 bp band
  - Negative Control shows no bands

Invalid Test Run

- A test run is invalid if:
  - The run has not been completed
  - Positive Control does not show the 238 bp band
  - Negative Control shows any amplification

![DNA Gel Image](image)

**Figure 1**: A representative 1X TAE 2% agarose gel showing the amplification of *Pneumocystis jirovecii*. The size of the *Pneumocystis jirovecii* target amplicon corresponds to the 238 bp band represented by the provided DNA Marker (M). No amplification of the target is observed in with the Negative Control

E. Specificity

The specificity of Norgen's *Pneumocystis jirovecii* PCR Kit is first and foremost ensured by the selection of the *P. jirovecii*-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies to all GenBank published sequences by sequence comparison analysis. The specific detectability of all relevant strains has thus been ensured by a database alignment and by PCR amplification with the following commonly-found pathogens: *Neisseria gonorrhoea, Chlamydia trachomatis*, Norovirus, West Nile Virus, HIV.
F. Linear Range

- The linear range (analytical measurement) of Norgen’s *Pneumocystis jirovecii* PCR Kit was determined by analyzing a dilution series of a *P. jirovecii* quantification standard ranging from $1 \times 10^7$ copies/µl to $1 \times 10^1$ copies/µl.
- Each dilution has been tested in replicates ($n = 4$) using Norgen’s *Pneumocystis jirovecii* PCR Kit on 1X TAE 1.7% Agarose gel.
- The linear range of Norgen’s *Pneumocystis jirovecii* PCR Kit has been determined to cover concentrations from $1 \times 10^2$ copies/µl to at least $1 \times 10^6$ copies/µl of isolated DNA.

G. Technical Support

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.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

Norgen’s *Pneumocystis jirovecii* PCR Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR and *in vitro* diagnostic procedures.

Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

The presence of PCR inhibitors may cause false negative or invalid results.

Potential mutations within the target regions of the *Pneumocystis jirovecii* genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.

The respective user is liable for any and all damages resulting from application of Norgen’s *Pneumocystis jirovecii* PCR Kit for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.