Cryptococcus neoformans PCR Kit
Product # 42720

Background Information

Cryptococcus neoformans is an encapsulated yeast. Infection with C. neoformans is known as cryptococcosis and is the cause of the most common life-threatening meningitis in patients with weakened immune systems, particularly in advanced HIV/AIDS. C. neoformans is commonly found in soil throughout the world. Human infection of C. neoformans occurs via inhalation of aerosolized spores. From the lungs, C. neoformans is spread hematogenously to the Central Nervous System (CNS), resulting in meningoencephalitis. Although the availability of antiretroviral therapy in the developed world has reduced the incidents of cryptococcosis, it is still a major problem in developing countries and is one of the leading causes of death in patients with HIV/AIDS. In fact, one of the major challenges in treating cryptococcosis is that many patients with cryptococcal CNS disease are asymptomatic in terms of cryptococcal pneumonia, making it difficult for early detection.

Product Description

Norgen’s Cryptococcus neoformans PCR Kit is a research use-only kit, based on the use of end-point PCR technology, for the detection of Cryptococcus neoformans specific DNA. The kit includes Master Mix and primers for the specific amplification of a 278 bp region of the Cryptococcus neoformans genome. In addition, the kit contains a positive and a negative control to confirm the integrity of the kit reagents.

The detection of Cryptococcus neoformans specific DNA is based on end-point PCR technology, utilizing polymerase chain reaction (PCR) for the amplification of specific Cryptococcus neoformans 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 Cryptococcus neoformans 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 # 42720 (48 preps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X PCR Master Mix</td>
<td>2 x 350 µL</td>
</tr>
<tr>
<td>C. neoformans Primer Set Mix</td>
<td>150 µL</td>
</tr>
<tr>
<td>C. neoformans 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>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

Storage Conditions and Product Stability

- The Cryptococcus neoformans 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 *Cryptococcus neoformans* 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. Do not use components of the kit that have passed their expiration date.
• The presence of PCR inhibitors may cause false negative or invalid results.
• Potential mutations within the target regions of the Cryptococcus neoformans 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 Cryptococcus neoformans 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 Cryptococcus neoformans 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 Cryptococcus neoformans 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 Cryptococcus neoformans 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>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-Free Water</td>
<td>8 µL</td>
</tr>
<tr>
<td>2X PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td><em>C. neoformans</em> Primer Set Mix</td>
<td>2 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></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><em>C. neoformans</em> Primer Set Mix</td>
<td>2 µL</td>
</tr>
<tr>
<td>Sample DNA</td>
<td>2.5 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 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><em>C. neoformans</em> Primer Set Mix</td>
<td>2 µL</td>
</tr>
<tr>
<td><em>C. neoformans</em> Positive Control (PosC)</td>
<td>5 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>

**C. Cryptococcus neoformans PCR Assay Programming**

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

<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. *Cryptococcus neoformans* 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 278 bp band corresponding to the *Cryptococcus neoformans* target amplicon
  - Positive Control shows the 278 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 278 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 278 bp band
  - Negative Control shows any amplification

**Figure 1:** A representative 1X TAE 2% agarose gel showing the amplification of *Cryptococcus neoformans*. The size of the *Cryptococcus neoformans* target amplicon corresponds to the 278 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 *Cryptococcus neoformans* PCR Kit is first and foremost ensured by the selection of the *C. neoformans*-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: *Pneumocystis jirovecii*, *Neisseria gonorrhoea*, *Chlamydia trachomatis*, Norovirus, West Nile Virus, HIV.

F. Linear Range
- The linear range (analytical measurement) of Norgen’s *Cryptococcus neoformans* PCR Kit was determined by analyzing a dilution series of a *C. neoformans* 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 *Cryptococcus neoformans* PCR Kit on 1X TAE 1.7% Agarose gel.
- The linear range of Norgen’s *Cryptococcus neoformans* 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
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 *Cryptococcus neoformans* 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 *Cryptococcus neoformans* 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.