**Cryptococcus neoformans** End-Point PCR Kit

**Product Insert**

**Intended Use**
Norgen’s *Cryptococcus neoformans* End-Point PCR Kit is designed for the detection of *Cryptococcus neoformans* DNA based on the use of end-point PCR technology. This kit is designed for research use only and not for use in diagnostic procedures.

**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* End-Point PCR Kit is designed for the detection of *Cryptococcus neoformans* specific DNA based on the use of end-point PCR technology. This kit is designed for research use only and not for use in diagnostic procedures. The kit includes Master Mix and primers for the specific amplification of a 278 nucleotide region of the *Cryptococcus neoformans* genome, as well as a positive control and a negative control to confirm the integrity of the kit reagents. In addition, the kit contains loading dye and a DNA ladder to facilitate analysis of the results.

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* End-Point PCR Kit was developed and validated to be used with the following PCR instruments:

- Qiagen Rotor-Gene Q
- BioRad CFX96 Touch™ Real-Time PCR Detection System

**Kit Components**

<table>
<thead>
<tr>
<th>Component</th>
<th>Product # EP42720 (24 preps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDx 2X PCR Master Mix</td>
<td>350 µL</td>
</tr>
<tr>
<td>Cryptococcus neoformans Primer Mix</td>
<td>70 µL</td>
</tr>
<tr>
<td>Cryptococcus neoformans Positive</td>
<td>50 µL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>Loading Dye</td>
<td>100 µL</td>
</tr>
<tr>
<td>DNA Ladder</td>
<td>100 µL</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>
Storage Conditions and Product Stability
- All kit components should be stored at -20°C upon arrival
- 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.
- All reagents can be stored for 1 year at -20°C without showing any reduction in performance.

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’s Blood DNA Isolation Mini Kit (Cat. 46300, 46380)
- 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
- PCR reaction preparation station (Optional)

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

Warnings and Precautions
- Norgen’s Cryptococcus neoformans End-Point PCR Kit is intended for research purposes only. It is not intended for diagnostic use.
- 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 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 been stored for more than 1 year.
- 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 End-Point PCR Kit. The quality of the DNA template will have a major impact on the performance of the Cryptococcus neoformans detection test. The user must ensure that the method used for DNA purification is compatible with end-point RT-PCR. We recommend the use of Norgen’s Blood DNA Isolation Mini Kit (Cat. 46300, 46380). Norgen’s Blood DNA Isolation Mini Kit has been fully validated with Norgen’s Cryptococcus neoformans End-Point PCR Kit.

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers is used, a column drying step consisting of centrifugation for 3 minutes at 20,000 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 MDx 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 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.
- 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 (i.e: 1) Nuclease-free water; 2) Master Mix; 3) Primer Mix; 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 1. PCR Negative Control Preparation**

<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><strong>MDx 2X PCR Master Mix</strong></td>
<td><strong>10 μL</strong></td>
</tr>
<tr>
<td>Cryptococcus neoformans Primer 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 2. PCR Cryptococcus neoformans Assay Preparation**

<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><strong>MDx 2X PCR Master Mix</strong></td>
<td><strong>10 μL</strong></td>
</tr>
<tr>
<td>Cryptococcus neoformans Primer 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 3. PCR Positive Control Preparation**

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDx 2X PCR Master Mix</strong></td>
<td><strong>10 μL</strong></td>
</tr>
<tr>
<td>Cryptococcus neoformans Primer Mix</td>
<td>2 μL</td>
</tr>
<tr>
<td>Cryptococcus neoformans Positive Control (PosC)</td>
<td>8 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 RT-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 1.4 % Agarose DNA gel along with 10 µL of Norgen’s DNA Ladder (provided).
- The PCR products should be resolved on the 1X TAE, 1.4 % Agarose gel at 150V for 30 minutes (Gel running time will vary depending on an electrophoresis apparatus).

Table 5. Interpretation of Assay Results

<table>
<thead>
<tr>
<th>Input Type</th>
<th>Target amplification Cryptococcus neoformans Target Band (278 bp)</th>
<th>PCR control Band (150 bp)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>Yes</td>
<td>Yes</td>
<td>Valid</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Yes</td>
<td>No</td>
<td>Valid</td>
</tr>
<tr>
<td>Negative Control</td>
<td>No</td>
<td>Yes</td>
<td>Valid</td>
</tr>
<tr>
<td>Sample</td>
<td>Yes</td>
<td>Yes</td>
<td>Positive</td>
</tr>
<tr>
<td>Sample</td>
<td>No</td>
<td>Yes</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample</td>
<td>No</td>
<td>No</td>
<td>PCR inhibition</td>
</tr>
<tr>
<td>Sample</td>
<td>Yes</td>
<td>No</td>
<td>Positive</td>
</tr>
</tbody>
</table>

For results obtained that are not covered in Table 5 above, please refer to the Frequently Asked Questions.
Figure 1: A representative 1X TAE, 1.4 % agarose gel showing the amplification of Cryptococcus neoformans at different concentrations. The size of the Cryptococcus neoformans target amplicon corresponds to the 278 bp band represented by the provided DNA Marker (M).

E. Cryptococcus neoformans PCR Assay Specificity

The specificity of Norgen’s Cryptococcus neoformans End-Point PCR Kit is first and foremost ensured by the selection of the Cryptococcus neoformans-specific primers, as well as the selection of stringent reaction conditions. The Cryptococcus neoformans primers were checked for possible homologies to all microorganisms in GenBank published sequences by sequence comparison analysis.

Frequently Asked Questions

1. How many samples should be included per PCR run?
   - Norgen’s Cryptococcus neoformans End-Point PCR 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 collect and test 6 samples at a time.

2. How should it be interpreted if in negative control the Cryptococcus neoformans PCR control and the Cryptococcus neoformans target showed amplification in a sample?
   - The assay has to be repeated. It could happen when there is carryover contamination.

3. How should it be interpreted if only the Cryptococcus neoformans target was amplified in a sample?
   - The sample tested should be considered as Cryptococcus neoformans positive. At high Cryptococcus neoformans input, the Cryptococcus neoformans amplicon will be predominant and thus the Cryptococcus neoformans PCR control may not amplify as they compete for PCR resources.
Related Products | Product #
---|---
Cryptococcus neoformans Primer and Control Set - 100 Reactions | EP42710
Cryptococcus neoformans TaqMan PCR Kit - 24 Reactions | TM42720
Cryptococcus neoformans TaqMan Probe/Primer and Control Set - 100 Reactions | TM42710
Blood DNA Isolation Mini Kit | 46300, 46380

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 Cryptococcus neoformans End-Point PCR Kit is designed for the detection of Cryptococcus neoformans specific DNA based on the use of end-point PCR technology. This kit is designed for research use only and not for use in diagnostic procedures.

Norgen’s Cryptococcus neoformans End-Point PCR Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR.

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 End-Point 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.

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