3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Pneumocystis jirovecii TaqMan PCR Kit Product Insert

Product # TM42850

Intended Use

Norgen's *Pneumocystis jirovecii* TaqMan PCR Kit is designed for the detection of *Pneumocystis jirovecii* specific DNA in a real-time PCR based on the use of TaqMan® technology. This kit is designed for research use only and not for use in diagnostic procedures.

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* TaqMan PCR Kit is designed for the detection of P*. jirovecii* specific DNA in a real-time PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures. The detection of *P. jirovecii* specific DNA is based on TaqMan PCR providing a simple, reliable and rapid result for the detection of *P. jirovecii* infection. Norgen's *Pneumocystis jirovecii* TaqMan PCR Kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result.

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

- Qiagen Rotor-Gene Q
- BioRad iCycler
- QuantStudio™ 7 Pro Real-Time PCR System

Kit Components

Component	Product # TM42850 (100 preps)
MDx TaqMan 2X PCR Master Mix	2 x 700 µL
P. jirovecii Primer & Probe Mix	280 µL
P. jirovecii Positive Control	150 µL
Nuclease-Free Water (Negative control)	1.25 mL
Product Insert	1

Storage Conditions and Product Stability

- The *Pneumocystis jirovecii* TaqMan *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 can be stored for 2 years after the date of production 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 TaqMan 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* TaqMan *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. TaqMan PCR Assay Preparation

Notes:

- Before use, suitable amounts of all TaqMan 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 TaqMan 2X PCR Master Mix provided is enough for up to 128 PCR reactions (96 sample PCR, 16 positive control PCR and 16 no template control PCR).
- For every TaqMan 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) MDx TaqMan 2X PCR Master Mix; 3) Primer & Probe 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

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	8 µL
MDx TaqMan 2X PCR Master Mix	10 µL
P. jirovecii Primer & Probe Mix	2 µL
Total Volume	20 µL

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 *Pneumocystis jirovecii* Assay Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	5.5 µL
MDx TaqMan 2X PCR Master Mix	10 µL
P. jirovecii Primer & Probe Mix	2 µL
Sample DNA*	2.5 µL
Total Volume	20 µL

* The recommended amount of sample DNA to be used is 3 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.

3. For each PCR set, prepare **one** positive control PCR as shown in Table 3 below:

Table 3. PCR Positive Control Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	3 µL
MDx TaqMan 2X PCR Master Mix	10 µL
P. jirovecii Primer & Probe Mix	2 µL
P. jirovecii Positive Control (PosC)	5 µL
Total Volume	20 µL

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 4. *Pneumocystis jirovecii* Assay Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95ºC**	**3 min**
Cycle 2 (40x)	**Step 1**	**95ºC**	**15 sec**
	Step 2	**60ºC**	**30 sec**

D. *Pneumocystis jirovecii* PCR Assay Interpretation

Table 5. Interpretation of Assay Results

FAM (Target detection)	HEX (PCR validation)	Result
+	**+**	**Positive**
-	**+**	**Negative**
-	**-**	**PCR inhibited**

For results obtained that are not covered in Table 5, please refer to the Frequently Asked Questions.

E. Specificity

The specificity of Norgen's *Pneumocystis jirovecii* TaqMan PCR Kit is first and foremost ensured by the selection of the *P. jirovecii*-specific primers and probe, as well as the selection of stringent reaction conditions. The primers and probe 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* TaqMan PCR Kit was determined by analyzing a dilution series of a *P. jirovecii* quantification standard ranging from 1 x 10^7 copies/µl to 1 x 10^{-1} copies/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen's *Pneumocystis jirovecii* TaqMan *PCR Kit* on 1X TAE 1.7% Agarose gel.
- The linear range of Norgen's *Pneumocystis jirovecii* TaqMan PCR Kit has been determined to cover concentrations from 1 x 10^2 copies/µl to at least 1 x 10^6 copies/µl of isolated DNA

Frequently Asked Questions

1. **How many samples should be included per PCR run?**
   - Norgen's *Legionella sp.* TaqMan PCR Detection Kit is designed to test 96 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 no PCR control signal (HEX) is detected while the target specific signal (FAM) is detected in the positive control?**
   - Tested samples(s) can be considered positive. It could happen when too much target DNA template was added due to the preferential amplification on the target.

3. **How should it be interpreted if the target specific signal (FAM) and/or the PCR control signal (HEX) are detected in the negative control?**
   - It could happen when there is carryover contamination and PCR inhibition. Repeat the assay using fresh aliquots and clean pipette tips.

4 **How should it be interpreted if no target signal (FAM) is detected in positive control?**
   - It could happen when the positive control was not added. Repeat the assay.

Related Products	Product #
Pneumocystis jirovecii TaqMan Probe/Primer and Control Set	TM64410
Blood Genomic DNA Isolation Mini Kit	46300

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 *Legionella sp*. TaqMan PCR Kit is designed for the detection of *Legionella sp.* specific DNA in a real-time PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures.

Norgen's *Legionella sp*. TaqMan 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 *Legionella sp*. 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 Legionella sp. TaqMan 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.

TaqMan is a registered trademark of Roche Molecular Systems, Inc

Norgen Biotek Corp.
3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

