Sputum-Based *Mycobacterium tuberculosis* PCR Detection Kit

Product # 42100

*Mycobacterium tuberculosis* is a pathogenic bacterial species belonging to the genus *Mycobacterium*, and is the causative agent of tuberculosis. Tuberculosis (TB) is a multifaceted disease and challenging public health problem in both industrialized and developing countries. According to the WHO, 8.8 million active cases of TB are diagnosed each year and of these, almost 2 million die. Once thought to be under control or even close to extinction, TB infection levels are rising and the threat is compounded by new, virulent and drug-resistant strains. Although most cases (~80%) occur in the developing world, increasing population mobility combined with the ease of transmission means that no country is immune from the resurgence of TB. TB control programs are currently facing a number of constraints. Worldwide, fewer than 25% of all tuberculosis cases are detected. Of utmost concern is the absence of a timely and accurate test for the diagnosis of mycobacterial disease. Early diagnosis is crucial for the prevention of further spread of the disease.

**Principle of the Test**

Norgen’s Sputum-Based *Mycobacterium tuberculosis* PCR Detection Kit is a ready-to-use system for the isolation and detection of *Mycobacterium tuberculosis* using end-point PCR. The kit first allows for the isolation of mycobacterial DNA from sputum samples using spin-column chromatography based on Norgen’s proprietary resin. The mycobacterial DNA is isolated free from inhibitors and can then be used as the template in a PCR reaction for detection using the provided *Mycobacterium tuberculosis* Master Mix. This Master Mix contains reagents and enzymes for the specific amplification of a 319 bp region of the *Mycobacterium tuberculosis* genome. In addition, Norgen’s Sputum-Based *Mycobacterium tuberculosis* 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. The 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>Binding Solution I</td>
<td>50 mL</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>0.6 mL</td>
</tr>
<tr>
<td>Binding Solution II</td>
<td>3.25 mL</td>
</tr>
<tr>
<td>Wash Solution</td>
<td>6.6 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>Mycobacterium tuberculosis 2X PCR Master Mix</strong></td>
<td>0.35 mL</td>
</tr>
<tr>
<td><strong>Control 2X PCR Master Mix</strong></td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Isolation Control (IsoC)</strong></td>
<td>0.3 mL</td>
</tr>
<tr>
<td><strong>Mycobacterium tuberculosis Positive Control (PosC)</strong></td>
<td>0.1 mL</td>
</tr>
<tr>
<td><strong>Nuclease-Free Water</strong></td>
<td>1.25 mL</td>
</tr>
<tr>
<td>Norgen’s DNA Marker</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

*IsoC = Isolation Control; PosC = Positive Control
a The isolation control is a cloned PCR product
b The positive control is a fragment of Mycobacterium tuberculosis cloned in a plasmid*
Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Centrifuge with a swinging bucket rotor capable of 2,000 RPM
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- Lysozyme solution (20 mg/mL)
- Dithiothreitol (100 µg/mL) or other solution for upstream sputum homogenization
- PCR tubes
- 96 – 100% ethanol
- 60°C incubator
- 15 mL conical tubes

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.

Norgen’s Sputum-Based Mycobacterium tuberculosis PCR Detection Kit contains ready-to-use Proteinase K solutions, which are dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

The Mycobacterium tuberculosis 2X PCR Master Mix, Control 2X PCR Master Mix, Isolation Control (IsoC), and Mycobacterium tuberculosis 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:

- All biological samples should be considered as potentially infectious. Proper biosafety measures should therefore be carried out when using this 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.

Quality Control

In accordance with Norgen’s ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen’s Mycobacterium tuberculosis 2X PCR Master Mix, Control 2X PCR Master Mix, Isolation Control (IsoC) and Mycobacterium tuberculosis Positive Control (PosC) are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen’s Sputum-based Mycobacterium tuberculosis 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 Binding Solution I, Binding Solution II and the Wash Solution 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 spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

1. Protocol

A. Specimen Collection, Storage and Transport

Precaution: All samples have to be treated as potentially infectious material.

1. Specimen Collection and Sample Storage
   - Expectorated or induced sputum samples may be collected
   - It is highly recommended that sputum samples be collected using Norgen’s Sputum DNA Collection, Preservation and Isolation Kit (Cat# 46100). The sputum samples can be stored for at least one year at room temperature when collected directly using Norgen’s Sputum DNA Collection, Preservation and Isolation Kit.
   - Alternatively, sputum samples collected using any other collection and preservation systems or reagents are also compatible with this kit.

2. Sample Transport
   - Sample material should be transported in a shatterproof, leak-proof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided.
   - The samples should be transported following the local and national instructions for the transport of pathogenic material.

B. Isolation of DNA from Sputum

Notes:
   - Do not spin down or filter the sputum sample before proceeding with the isolation, as this could negatively affect the isolation of DNA.
   - 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.
   - Always vortex the Proteinase K solution before use.
   - Preheat an incubator or heating block to 60°C.
   - Prepare a working concentration of Binding Solution I, Binding Solution II and Wash Solution by adding the appropriate volume of 96-100% ethanol to the supplied bottles containing the concentrated solutions (see Table 1 below). The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
   - Prior to the DNA isolation it is recommended that the viscous sputum sample be liquefied. This can be accomplished by adding a reducing agent such as dithiothreitol to the sample and heating at 37°C for 20 minutes to completely homogenize the sample. We recommend preparing a solution of DTT at a concentration of 100 µg/mL and then adding an equal volume to the sputum sample to give a final concentration of 50 µg/mL.

Table 1. Volume of Ethanol to be added to Binding Buffer I, Binding Buffer II and Wash Buffer

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume Provided</th>
<th>Ethanol (96-100%) Volume to be Added by User</th>
<th>Final Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding Solution I</td>
<td>50 mL</td>
<td>50 mL</td>
<td>50 mL</td>
</tr>
<tr>
<td>Binding Solution II</td>
<td>3.25 mL</td>
<td>3.25 mL</td>
<td>6.5 mL</td>
</tr>
<tr>
<td>Wash Solution</td>
<td>6.6 mL</td>
<td>18.4 mL</td>
<td>25 mL</td>
</tr>
</tbody>
</table>
**Isolation Control (IsoC)**
- A 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.

1. Add 4 mL of Binding Solution I for 1 mL of sputum sample. Mix well by pipetting up and down several times. *(Note: Binding Solution I contains resin and must be mixed well before every pipetting).*
2. Centrifuge for 5 minutes at 2,000 RPM and discard the supernatant.
3. Add 20 μL of both Proteinase K and Lysozyme (user supplied) to the precipitated slurry pellet resulting from the sputum sample. Vortex for 10 seconds.
4. Incubate the mixture at 60°C for 20 minutes.
5. After the 20 minute incubation, add 260 μL of Binding Solution II.
6. Centrifuge for 1 minute at 10,000 RPM, and discard the flow-through.
7. Apply 500 µL of Wash Solution to the column and centrifuge for 1 minute at 14,000 RPM. Discard the flow-through and reassemble the spin column with its collection tube.
8. Apply 500 µL of Wash Solution to the column and centrifuge for 1 minute at 14,000 RPM. Discard the flow-through and reassemble the spin column with its collection tube.
9. Apply 500 µL of 95% ethanol and centrifuge for 1 minute at 14,000 RPM. Discard the flow-through and reassemble the spin column with its collection tube.
10. Spin the column for 2 minutes at 14,000 RPM in order to thoroughly dry the resin. Discard the collection tube.
11. Incubate the column horizontally with the lid open at 60°C for 10 minutes.
12. 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 2,000 RPM, followed by a 1 minute spin at 14,000 RPM.

C. *Mycobacterium tuberculosis* 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 *Mycobacterium tuberculosis* 2X Detection 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 *Mycobacterium tuberculosis* 2X Detection 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 *Mycobacterium tuberculosis* 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 from the sample than recommended may affect the sensitivity of the *Mycobacterium tuberculosis* Limit of Detection.

1. Prepare the PCR reaction for sample detection (Set #1, using *Mycobacterium tuberculosis 2X Detection PCR Mastermix*) and the PCR reaction for control detection (Set #2, using Control 2X PCR Mastermix) 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 Malaria 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 1. PCR Assay Preparation

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

2. For each PCR set, prepare one positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium tuberculosis 2X PCR Master Mix OR Control 2X PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>Malaria Positive Control (PosC)</td>
<td>10 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

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

Table 3. PCR Negative Control Preparation

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

D. Mycobacterium tuberculosis PCR Assay Programming
1. Program the thermocycler according to the program shown in Table 4 below.
2. Run one step PCR.

Table 4. Mycobacterium tuberculosis 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. *Mycobacterium tuberculosis* 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 Marker (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).

![Figure 1](image1.png)

**Figure 1:** A representative 1X TAE, 1.7% agarose gel showing the amplification of *Mycobacterium tuberculosis* at different concentrations. The size of the *Mycobacterium tuberculosis* target amplicon corresponds to the 319 bp band represented by the provided DNA Marker (M). Lanes 1-8 represent samples spiked with different *Mycobacterium tuberculosis* concentrations isolated from 1 mL sputum samples (interpreted as positive results). The *Mycobacterium tuberculosis* spiked in sputum samples is purified plasmid DNA.

![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. NTC=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>Mycobacterium</td>
<td>IsoC Band (499bp)</td>
<td></td>
</tr>
<tr>
<td>tuberculosis</td>
<td>tuberculosis</td>
<td>PCRC Band (150 bp)</td>
<td></td>
</tr>
<tr>
<td>Target Band (319 bp)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>X</td>
<td>X</td>
<td>Valid</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td>X</td>
<td>Valid</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
<td>Positive</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td>X</td>
<td>Re-Test</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td>X</td>
<td>Positive</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td></td>
<td>Re-Test</td>
</tr>
</tbody>
</table>

** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

** Ignore any bands that appear between the Isolation Control band and the PCR Control band

E. Specificity

The specificity of Norgen’s Sputum-Based *Mycobacterium tuberculosis* PCR Detection Kit is first and foremost ensured by the selection of the *Mycobacterium tuberculosis*-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies in GenBank published sequences by sequence comparison analyses.

F. Linear Range

- The linear range (analytical measurement) of Norgen’s Sputum-Based *Mycobacterium tuberculosis* PCR Detection Kit was determined by analyzing a dilution series of *Mycobacterium tuberculosis* quantitative standard ranging from $8.46 \times 10^9$ copies/µl to $1 \times 10^{-1}$ copies/µl.
- Each dilution has been tested in replicates ($n = 4$) using Norgen’s Sputum-Based *Mycobacterium tuberculosis* PCR Detection Kit on 1X TAE, 1.7% Agarose gels.
- The linear range of Norgen’s Sputum-Based *Mycobacterium tuberculosis* PCR Detection Kit has been determined to cover concentrations from 2 copies/µl to at least $8 \times 10^6$ copies/µl.
- Under the conditions of Norgen’s Sputum DNA Isolation procedure, Norgen’s Sputum-Based *Mycobacterium tuberculosis* PCR Detection Kit covers a linear range from 1000 copies/mL sputum to at least $8 \times 10^6$ copies/mL sputum.
G. Frequently Asked Questions

1. How many samples should be included per PCR run?
   - Norgen’s Sputum-Based *Mycobacterium tuberculosis* PCR Detection Kit is designed to test 24 samples. For every 6 samples, a Negative Control and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Negative Control and Positive Control are enough to run 3 samples at a time.

2. How can I interpret my results for a sample if neither the *Mycobacterium tuberculosis* PCR control nor the *Mycobacterium tuberculosis* Isolation Control (IsoC) amplifies?
   - If neither the *Mycobacterium tuberculosis* PCR control nor the *Mycobacterium tuberculosis* 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 *Mycobacterium tuberculosis* PCR control showed amplification but neither the *Mycobacterium tuberculosis* target nor the *Mycobacterium tuberculosis* Isolation Control (IsoC) amplified for a sample?
   - This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the *Mycobacterium tuberculosis* Isolation Control (IsoC) was amplified in a sample?
   - The sample tested can be considered as *Mycobacterium tuberculosis* negative.

5. How should it be interpreted if only the *Mycobacterium tuberculosis* target and the *Mycobacterium tuberculosis* PCR control were amplified in a sample?
   - The sample tested can be considered as *Mycobacterium tuberculosis* positive.

6. How should it be interpreted if only the *Mycobacterium tuberculosis* target was amplified in a sample?
   - The sample tested can be considered positive. At high *Mycobacterium tuberculosis* concentration, the *Mycobacterium tuberculosis* amplicon will be predominant and the *Mycobacterium tuberculosis* PCR control as well as the *Mycobacterium tuberculosis* Isolation control may not amplify.

7. How should it be interpreted if only the *Mycobacterium tuberculosis* PCR control and the *Mycobacterium tuberculosis* Isolation Control (IsoC) showed amplification?
   - The sample tested can be considered negative.

8. Can I process a different sputum volume?
   - The reagents provided with the isolation kit are only sufficient to process 24 sputum samples of 1 mL each.

9. What If I added more or less of the specified reagents’ volume during DNA isolation?
   - Adding less volume may reduce your DNA yields. Adding more may not affect the DNA yields EXCEPT if more Elution Buffer was added. Eluting DNA in higher volumes of Elution Buffer will result in diluting your DNA.

10. What If my incubation temperature varied from the specified 60°C?
    - The incubation temperature can be in the range of 55°C - 65°C. At other temperatures the activity of the Proteinase K will be reduced. This will result in a reduction in your DNA yields.

11. What If my incubation varied from the 20 minutes specified in the product manual?
    - Less than 20 minutes will result in lower DNA yields. More than 20 minutes may not affect your DNA yields.

12. What If I forgot to do a dry spin after my second wash?
    - Your DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your elution and it may interfere with your downstream applications.

13. What If I forgot to add the *Mycobacterium tuberculosis* Isolation control during the Isolation?
    - The Isolation must be repeated.
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 Sputum-based Mycobacterium tuberculosis 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.