Hepatitis C virus infection is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) worldwide. Neither a vaccine nor an effective treatment is available for HCV. Pegylated interferon-α (PEG-IFN-α), combined with ribavirin, is the current standard therapy. Its efficiency ranges between 20-80%, depending on the HCV genotype. Unfortunately, many HCV infected patients do not respond to or do not tolerate the IFN-based therapy. Therefore, the number of patients progressing to HCC as a result of HCV infection is expected to increase over the next 20-30 years. The natural history of the disease reveals the elusive nature of the virus in a number of features. First, the infection is often detected incidentally at the time of blood donation as the acute infection is clinically asymptomatic in most patients. Second, HCV successfully escapes multi-specific host immune responses in the majority of patients which establishes persistent infection. Third, a significant number of persistently infected individuals remain unaware of the infection for decades, until liver fibrosis, cirrhosis and/or hepatocellular carcinoma develop.

Principle of the Test
Norgen’s Plasma/Serum HCV RT-PCR Detection Kit constitutes a ready-to-use system for the isolation and detection of HCV viral RNA using end-point RT-PCR. The kit first allows for the isolation of circulating RNA, including viral RNA, from the plasma/serum samples using Norgen’s proprietary resin as the separation matrix. The viral RNA is isolated free from inhibitors, and can then be used as the template in a RT-PCR reaction for HCV detection using the provided HCV Master Mix. The HCV Master Mix contains reagents and enzymes for the specific amplification of a 380 bp region of HCV. In addition, Norgen’s Plasma/Serum HCV RT-PCR Detection Kit contains a second heterologous amplification system to identify possible PCR inhibition and/or inadequate isolation. The amplification and detection of either the HCV Isolation Control (IsoC) or the PCR control (PCRC) does not reduce the detection limit of the analytical HCV PCR. The kit is designed to allow for the testing of 12 samples.

Kit Components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Solution</td>
<td>40 mL</td>
</tr>
<tr>
<td>Wash Solution I</td>
<td>5.5 mL</td>
</tr>
<tr>
<td>Elution Solution I</td>
<td>10 mL</td>
</tr>
<tr>
<td>Binding Solution</td>
<td>15 mL</td>
</tr>
<tr>
<td>Wash Solution II</td>
<td>4 mL</td>
</tr>
<tr>
<td>Elution Solution II</td>
<td>2 mL</td>
</tr>
<tr>
<td>Maxi Filter Columns</td>
<td>12</td>
</tr>
<tr>
<td>Mini Spin Columns with Collection Tubes</td>
<td>12</td>
</tr>
<tr>
<td>Elution tubes (1.7 mL)</td>
<td>12</td>
</tr>
<tr>
<td><strong>HCV 2x RT-PCR Master Mix</strong></td>
<td>0.2 mL</td>
</tr>
<tr>
<td><strong>HCV Isolation Control (IsoC)</strong></td>
<td>0.2 mL</td>
</tr>
<tr>
<td><strong>HCV Positive Control (PosC)</strong></td>
<td>0.2 mL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>Norgen's DNA Marker</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

a The positive control is an in vitro transcribed HCV RNA fragments.
b The isolation control is a cloned PCR product.
Customer-Supplied Reagents and Equipment

- Disposable Latex gloves
- Centrifuge with a swinging bucket rotor capable of 2000 x g and Benchtop microcentrifuge
- Micropipettes with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
- Laminar flow hood for extractions
- Vortex and Microcentrifuge tube rack
- Sterile, nuclease-free aerosol-barrier micropipettor tips and PCR tubes
- 96 – 100% ethanol and β-mercaptoethanol
- 50 mL conical tubes

Storage Conditions and Product Stability

- The Positive Control (HCV PosC, red cap) and Isolation Control (HCV IsoC, orange cap) should be stored at -70°C. If needed, make aliquots of the controls according to the volume used in the protocol (10 µL of HCV PosC or 15 µL of HCV IsoC) prior to freezing.
- The HCV 2X RT-PCR Mastermix should be stored at -20°C. Make appropriate aliquots if needed
- All other kit components may be stored at room temperature
- The HCV 2X RT-PCR Mastermix, HCV Postive Control (PosC) and HCV Isolation Control (IsoC) should not undergo repeated freeze-thaw (a maximum freeze-thaw of three times).
- Allow reagents to thaw at room temperature prior to use
- After addition of samples to RT-PCR Master Mix use within one hour
- Kit reagents are stable through the end of the expiration month indicated on the packaging label when stored at the recommended temperatures.

General Precautions

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Diagnostic laboratory work on clinical samples from patients who are suspected of having HCV infection should be conducted in a BSL2 laboratory. All sample manipulations should be carried out in a biosafety cabinet. Viral isolation on clinical specimens from patients who are suspected of having HCV infection should be performed in a BSL2 laboratory with BSL3 practices
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Workflow in the laboratory should proceed in a uni-directional manner, beginning in the pre-amplification area(s) (i.e. specimen collection and RNA extraction) and moving to the amplification / detection area(s) (RT-PCR and gel electrophoresis).
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Supplies and equipment used for specimen preparation should not be used for pipetting or processing amplified RNA or other sources of target nucleic acids.
- All amplification supplies and equipment should be kept in the amplification / detection area at all times.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- As contamination of patient 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.
- Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Do not interchange reagent tube / bottle caps as this may lead to contamination and compromise test results.
• Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.

Quality Control
In accordance with Norgen’s ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen’s Plasma/Serum HCV RT-PCR Detection Kit, the HCV 2x RT-PCR Master Mix, the HCV Isolation Control (IsoC), the HCV Negative Control (NegC) and the HCV Positive Control (PosC) are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen’s Plasma/Serum HCV RT-PCR Detection Kit is designed for research purposes only.

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
Biosafety level 2 practices are recommended for works involving clinical samples from patients who are suspected of having HCV infection. Ensure the appropriate containment equipment and facilities are used for activities involving cultures or potentially infectious clinical materials. 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 Lysis Solution contains guanidine salts, and should be handled with care. Guanidine salts form 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 spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Protocol

A. Specimen Collection, Storage and Transport
Precaution: All samples must be treated as potentially infectious material.

1. Specimen Collection and Sample Storage
   • Blood withdrawal causes injury of blood vessels (arteries, veins and capillaries).
   • Only safe and sterile material should be used.
   • For blood withdrawal appropriate disposables are available. For the vein puncture, too fine capillary needles should not be employed.
   • Venous blood withdrawal should be carried out on the appropriate parts of the elbow bend, the forearm or the back of the hand.
   • Blood has to be withdrawn with standard specimen collection tubes (red cap, Sarstedt or equivalent tube of another manufacturer). 5 - 10 ml EDTA blood should be withdrawn.
   Precaution: Samples of heparinised humans must not be used

2. Sample Storage
   • Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800 - 1,600 x g within six hours. The isolated plasma has to be transferred into sterile polypropylene tubes.
   • The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time.
• Virus encapsulated DNA is stable for days if stored at +4 °C, for weeks if stored at -20 °C and even for months and years when stored at -70 °C.

3. 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 pathogen material.
• We recommend sample transport with a courier. The blood samples should be shipped cooled (+2 °C to +8 °C) and the separated plasma deep frozen (-20 °C).

4. Interfering substances
• Elevated levels of bilirubin (15 mg/dl) and lipids (800 mg/dl) and haemolytic samples do not influence the system.
• Heparin (10 IU/ml) affects the PCR. Samples which have been collected in tubes containing heparin as an anticoagulant should not be used. Also, samples of heparinised patients must not be used.

B. Purification of Circulating and Viral RNA from 2mL Serum or Plasma
Notes:
• All centrifugation steps are carried out in a benchtop microcentrifuge at 6,700 x g (~10,000 RPM) except where noted.
• All centrifugation steps are performed at room temperature.
• A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
• Ensure that all solutions are at room temperature prior to use.
• Prepare a working concentration of the Wash Solution I by adding 14.5 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 20 mL. The bottle label contains a box to check to indicate that the ethanol has been added.
• Prepare a working concentration of the Wash Solution II by adding 10 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 14 mL. The bottle label contains a box to check to indicate that the ethanol has been added.
• The use of β-mercaptoethanol during lysis is highly recommended to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of RNA Lysis Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
• If any precipitates are observed in the Lysis Solution or Binding Solution it is recommended to warm the solutions for 20 minutes at 60 °C.
• It is important to work quickly during this procedure.
• An HCV Isolation Control (IsoC) is supplied. This allows the user to control the viral RNA isolation procedure. For this assay, add the HCV Isolation Control (IsoC) to the lysate during the isolation procedure.
  o The HCV Isolation Control (IsoC) must not be added to the sample material directly
  o Do not freeze and thaw the HCV Isolation Control (IsoC) more than 3 times.
  o The HCV Isolation Control (IsoC) must be kept on ice at all times during the isolation procedure.

1. Add 3 mL of Lysis Solution (after the addition of β-mercaptoethanol) to a 2 mL plasma/serum sample. Vortex for 15 seconds.
2. Add 3 mL of 95 - 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.
3. Add 15 µL of HCV Isolation Control (IsoC) to the lysate. Vortex for 10 seconds.
4. Transfer up to 4 mL of lysate to the provided Maxi Column assembly and securely close the lid. **Vortex the column assembly for 15 seconds. Do Not Over Vortex.**
   o **Note:** During vortexing, the grey resin that is contained within the column will mix with the lysate to form grey colored slurry.
5. After the vortexing step centrifuge the Maxi Column assembly for 2 minutes at 2,000 RPM. Remove the collection tube and discard the flowthrough. Reassemble the Maxi Column with the collection tube.
6. Repeat **Steps 4 and 5** in order to process the entire lysate volume
7. After the entire lysate volume has been applied to the Maxi Column, apply 700 µL of Wash Solution I to the column and centrifuge for 1 minute at 2,000 RPM. Discard the flowthrough and reassemble the Maxi Column with the collection tube.
8. Repeat **Step 7** a second time.
9. Spin the column, empty, for 2 minutes at 3,000 RPM. Discard the collection tube.
10. Transfer the spin column to a fresh 50 mL conical tube (not provided). Apply 700 µL of Elution Solution I to the column and centrifuge for 2 minutes at 2000 RPM, followed by 3 minutes at 3,000 RPM. Remove the Maxi Column from the conical tube and discard.
11. Add 1 mL of **Binding Solution** to the eluted RNA contained within the conical tube from **Step 10** above. **Vortex for 15 seconds.**
12. Add 1 mL of 95 – 100% ethanol (provided by the user) to the mixture from **Step 10**. Mix by vortexing for 10 seconds.
13. Apply up to 700 µL of the mixture from **Step 11** onto the provided Mini Spin Column and centrifuge for 1 minute at 14,000 RPM. Discard the flowthrough. Reassemble the spin column with its collection tube.
14. Repeat **Step 13** as necessary to load the rest of the sample that results from **Step 12**.
15. Apply 400 µL of **Wash Solution II** to the column and centrifuge for 1 minute at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.
16. Repeat **Step 15** to wash column a second time.
17. Spin the column for 2 minutes at 14,000 RPM in order to thoroughly dry the resin. Discard the collection tube.
18. Place the column into a fresh 1.7 mL Elution tube provided with the kit. Add 100 µL of **Elution Solution II** to the column. Centrifuge for 2 minutes at 2,000 RPM, followed by 1 minute at 14,000 RPM.
   - **Note** the volume eluted from the column. If the entire 100 µL has not been eluted, spin the column at 14,000 RPM for 1 additional minute.

**C. HCV RT-PCR Assay Preparation**

**Notes:**
- It is recommended that 10 µL of the RNA elution be used as the RT-PCR sample input volume.
- Sample volume can be varied between 2 µL – 10 µL of the RNA elution. PCR grade water should be added to make up the final volume of the RT-PCR reaction to 20 µL.
- Using a lower volume from the sample than recommended may affect the sensitivity of the HCV Limit of Detection.
- A **Negative Control** (Nuclease-Free Water and HCV Positive Control (PosC)) must be included during every run.
- The **HCV Negative Control** (NegC) and **HCV Positive Control** (PosC) provided are sufficient for eight PCR runs.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or quick vortexing), and centrifuged briefly.

1. Prepare RT-PCR reactions as outlined in Table 1 below. For each sample to be run, pipette 10 µL of the eluted RNA and 10 µL of the Master Mix into a PCR tube. Each RT-PCR reaction will have a final volume of 20 µL.
2. Pipette 10 µL of **Nuclease-Free Water** into a PCR tube and add 10 µL of Master Mix. Pipette 10 µL of **HCV Positive Control** (PosC) into a PCR tube and add 10 µL of Master Mix.
3. Program the PCR machine according to the program shown in Table 2 below.
4. Run PCR.
Table 1. PCR Assay Preparation

<table>
<thead>
<tr>
<th>Preparation of RT-PCR assay</th>
<th>Volume Per RT-PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV 2X RT-PCR Master Mix</td>
<td>10 µL 10 µL 10 µL</td>
</tr>
<tr>
<td>Sample (Eluted RNA)</td>
<td>10 µL</td>
</tr>
<tr>
<td>HCV Positive Control (PosC)</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 20 µL 20 µL</td>
</tr>
</tbody>
</table>

1. Program the thermocycler according to the program shown in Table 2 below.
2. Run RT-PCR.

Table 2. HCV RT-PCR 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>50°C</td>
<td>30 min</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>Step 1</td>
<td>95°C</td>
<td>3 min</td>
</tr>
<tr>
<td>Cycle 3 (40x)</td>
<td>Step 1</td>
<td>94°C</td>
<td>15 sec</td>
</tr>
<tr>
<td></td>
<td>Step 2</td>
<td>64°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 4</td>
<td>Step 1</td>
<td>72°C</td>
<td>5 min</td>
</tr>
<tr>
<td>Cycle 5</td>
<td>Step 1</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

D. HCV 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
- Figure 1 and Table 3 explain how to interpret the PCR assay results
Figure 1: A representative 1X TAE, 1.7% agarose gel showing the amplification of HCV at different concentrations (Target). The size of the HCV target amplicon corresponds to the 380 bp band represented by the provided DNA Marker (M). The size of the HCV Isolation Control (IsoC) corresponds to the 500 bp band represented by the provided DNA Marker (M). The HCV 2X RT-PCR Master Mix contains a HCV PCR Control (PCRC). The HCV PCRC Controls for PCR inhibition. The size of the HCV PCRC corresponds to the 150 bp band represented by the provided DNA Marker (M). Lanes A-H represents samples spiked with different HCV concentrations isolated from 2 mL plasma samples (interpreted as positive results). The HCV spiked in plasma samples is an in vitro transcribed HCV RNA fragments.

Table 3. Possible outcomes for the interpretation of PCR assay results

<table>
<thead>
<tr>
<th>Input Type</th>
<th>HCV IsoC Band (500 bp)</th>
<th>HCV Target Band (380 bp)</th>
<th>HCV PCRC Band (150 bp)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Valid</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td>X</td>
<td></td>
<td>Valid</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Positive</td>
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<tr>
<td>Sample</td>
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<td></td>
<td>X</td>
<td>Negative</td>
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<td>Sample</td>
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<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td>X</td>
<td>Positive</td>
</tr>
</tbody>
</table>

** For results obtained that are not covered in Table 3 above, please refer to the Troubleshooting Section.

E. Specificity

The specificity of Norgen’s Plasma/Serum HCV RT-PCR Detection Kit is first and foremost ensured by the selection of the HCV-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. Furthermore, the specificity of the HCV-specific primers were tested against most of the known sexually-transmitted pathogens.
F. Linear Range

- The linear range of Norgen’s Plasma/Serum HCV RT-PCR Detection Kit was determined by analyzing a dilution series of HCV quantitative standards ranging from $8.46 \times 10^9$ VP/µl to $1 \times 10^7$ IU/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen’s Plasma/Serum HCV RT-PCR Detection Kit on 1X TAE, 1.7% Agarose gels.
- The linear range of Norgen’s Plasma/Serum HCV RT-PCR Detection Kit has been determined to cover concentrations from $12$ VP/µl to at least $8 \times 10^6$ VP/µl.
- Under the conditions of Norgen’s Plasma/Serum circulating RNA Isolation procedure, Norgen’s Plasma/Serum HCV RT-PCR detection Kit covers a linear range from $200$ VP/mL Plasma/Serum to at least $8 \times 10^6$ VP/mL Plasma/Serum.

G. Frequently Asked Questions

1. How many samples should be included per PCR run?
   - Norgen’s Plasma/Serum HCV RT-PCR Detection Kit is designed to test 12 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 HCV PCR control nor the HCV Isolation Control (IsoC) amplifies?
   - If neither the HCV PCR control nor the HCV 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 HCV PCR control showed amplification but neither the HCV target nor the HCV 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 HCV Isolation Control (IsoC) was amplified in a sample?
   - The sample tested can be considered as HCV negative.

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

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

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

8. Can I process a different Plasma/Serum volume?
   - The reagents provided with the isolation kit are only sufficient to process 12 Plasma/Serum samples of 2mL each.

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

10. What if I forgot to do a dry spin after my second wash?
    - Your RNA elution will be contaminated with the Wash Solution. This may dilute the RNA yield in your elution and it may interfere with your downstream applications.
11. What If I forgot to add the HCV 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 Plasma/Serum HCV 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.