NORGEN BIOTEK CORPORATION

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

Parvo B19 TaqMan PCR Kit Dx

Product Insert

REF **DxTM39600** CE IVD PIDxTM39600-3

Intended Use

Norgen's Parvo B19 TaqMan PCR Kit Dx is an *in vitro* diagnostic test for the detection and quantification of Parvovirus 19 specific DNA using real-time hybridization-fluorescence detection.

Background Information

Parvovirus B19 was the first known human virus in the family of parvoviruses, genus erythrovirus. B19 virus causes a childhood rash called fifth disease or erythema infectiosum which is commonly called "slapped cheek" syndrome due to the appearance of erythema across the cheeks. B19 is a non-enveloped, icosahedral virus that contains a single-stranded linear DNA genome. It is classified as an erythrovirus because of its capability to invade red blood cell precursors in the bone marrow. The virus is primarily spread by infected respiratory droplets. Blood-borne transmission, however, has been reported. Parvovirus infection in pregnant women is associated with hydrops fetalis due to severe fetal anemia, sometimes leading to miscarriage or stillbirth. The risk of fetal loss is about 10% if infection occurs before week 20 of pregnancy. There is also some evidence that intrauterine Parvovirus B19 infection leads to developmental abnormalities in childhood. Due to the serious nature of the virus, methods for its rapid diagnosis are desirable.

Product Description

Norgen's Parvo B19 TaqMan PCR Kit Dx is an *in vitro* diagnostic test for the detection and quantification of Parvo B19 specific DNA using real-time hybridization-fluorescence detection. The kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The Parvo B19 TaqMan PCR Kit Dx comprises Master Mix for the target and PCR control detection, Primer & Probe Mix, as well as a positive control and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.

Norgen's Parvo B19 TaqMan PCR Kit Dx is based on real-time PCR technology, utilizing PCR for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes. Probes specific for Parvo B19 are labelled with the fluorophore FAM. The probe specific for the PCR control is labelled with the fluorophore HEX. Using probes linked to distinguishable dyes enables the parallel detection of Parvo B19 specific DNA and the PCR control in corresponding detector channels of the real-time PCR instrument.

The supplied positive control can be used individually, or it can be diluted to generate a standard curve that can be used to determine the concentration of Parvo B19 in the sample. To generate the standard curve the following concentrations are used:

Standard Sample	Copy Number Per 5 µL	Note
A	1.00E+06	Supplied concentration
B	1.00E+05	Dilution
C	1.00E+04	Dilution
D	1.00E+03	Dilution
E	1.00E+02	Dilution
F	1.00E+01	Dilution

Norgen's Parvo B19 TaqMan PCR Kit Dx was developed and validated to be used with the following real-time PCR instruments:

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

Kit Components

Component	Product # DxTM39600 (24 preps)
MDx TaqMan 2X PCR Master Mix Dx	550 µL
Parvo B19 Primer & Probe Mix Dx	2 x 70 µL
Parvo B19 Positive Control Dx – 200,000 copies/µL	50 µL
Nuclease-Free Water	1.25 mL
Product Insert	1

Label Legend

Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests	Manu-facturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temper-ature limitation

Storage Conditions and Product Stability

- The Parvo B19 TaqMan PCR Kit Dx 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
- Repeated thawing and freezing (> 3 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 used until the expiration date specified on their labels.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Parvo B19 TaqMan PCR Kit Dx is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Norgen's Parvo B19 TaqMan PCR Kit Dx is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR and *in vitro* diagnostic procedures.
- Follow universal precautions. All patient 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 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.
- 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.
- As with any diagnostic test, results generated using Norgen's Parvo B19 TaqMan PCR Kit Dx should be interpreted with regard to other clinical or laboratory findings.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the Parvo B19 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.

Customer-Supplied Reagents and Equipment

- Appropriate Real-Time PCR Instrument with FAM and HEX filter channel
- 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 Dx series of purification kits for DNA isolation, including:
        - Norgen's Blood Genomic DNA Isolation Mini Kit Dx - Cat# Dx46300
        - Norgen's Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format) - Cat# Dx50600
        - Norgen's Urine DNA Isolation Kit Dx (Slurry Format) - Cat# Dx48800
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- PCR tube centrifuge
- PCR reaction preparation station (Optional)

Procedure

A. Sample Preparation

Purified DNA is the starting material for Norgen's Parvo B19 TaqMan PCR Kit Dx. The quality of the DNA template will have a major impact on the performance of the diagnostic test. The user must ensure that the method used for DNA purification is compatible with PCR technology. We recommend the use of Norgen's Dx series of purification kits for DNA isolation, including **Norgen's Blood DNA Isolation Mini Kit Dx (Cat# Dx46300)**, **Norgen's Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format) (Cat# Dx50600)** or **Norgen's Urine DNA Isolation Kit Dx (Slurry Format) (Cat# Dx48800).** Norgen's Dx series of purification kits have been fully validated with Norgen's TaqMan PCR kits for *in vitro* diagnostic testing.

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 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. TaqMan PCR Assay Preparation

Notes:

- Before use, suitable amounts of all TaqMan PCR components should be completely thawed, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
- Work quickly on ice.
- The recommended minimum number of DNA samples tested per TaqMan PCR run is 8.
- The amount of MDx TaqMan 2X PCR Master Mix Dx provided is enough for up to 45 PCR reactions (3 x 8 sample PCR reactions, 3 x 6 positive control serial dilution PCR reactions and 3 no template control PCR reactions).
- For every TaqMan PCR run, one reaction containing Parvo B19 Positive Control (or optionally 6 reactions for the provided positive control and 5 serial dilutions for qualitative PCR) and one reaction as no template control must be included for proper interpretation of results.
- For quantitative PCR, a standard curve should be generated using the provided Positive Control and 5 dilutions from the positive control (6 PCR reactions).

i. Reaction Setup for Qualitative Analysis

1. Prepare the Reaction Master Mix by combining the PCR components in a clean tube according to the following pipetting scheme:

PCR component	1 reaction	*11 reactions
Nuclease-Free Water	3 µL	33 µL
MDx TaqMan 2X PCR Master Mix	10 µL	110 µL
Parvo B19 Primer & Probe Mix	2 µL	22 µL
Total Volume	**15 µL**	**165 µL**

* The volume of the Reaction Master Mix depends on the number of samples to be prepared.

2. Mix well by pipetting or gentle vortexing. Spin briefly to collect any liquid in the tube cap.
3. Pipette 15 µL of the Reaction Master Mix into the appropriate PCR tubes.
4. Add 5 µL of the Negative Control, Sample or Positive Control, and pipette to mix. A minimum of one positive control and one negative control wells should be used.

5. Cap the tubes with the appropriate optical lid and spin briefly at 3000 RPM for a few seconds.

Note: To avoid any contamination while adding the templates, ensure that they are added in the follow the order:

1. Negative Control
2. Test Sample
3. Positive Control

ii Reaction Setup for Quantitative Analysis

1. Prepare the Reaction Master Mix by combining the PCR components in a clean tube according to the following pipetting scheme:

PCR component	1 reaction	*16 reactions
Nuclease-Free Water	3 µL	48 µL
MDx TaqMan 2X PCR Master Mix	10 µL	160 µL
Parvo B19 Primer & Probe Mix	2 µL	32 µL
Total Volume	**15 µL**	**240 µL**

* The volume of the Reaction Master Mix depends on the number of samples to be prepared.

2. Mix well by pipetting or gentle vortexing. Spin briefly to collect any liquid in the tube cap.
3. Pipette 15 µL of the Reaction Master Mix into the appropriate PCR tubes.
4. Add 5 µL of the Negative Control or Sample, and pipette to mix. A minimum of one negative control well should be used.
5. Cap the tubes with the appropriate optical lid and spin briefly at 3000 RPM for a few seconds.
6. Prepare a serial dilution of the Positive Control Dx (200,000 copies/µL), according to the following steps:
   a. Prepare 5 clean tubes containing 45 µL of Nuclease-Free water.
   b. Use 5 µL of the Positive Control Standard supplied with the kit (200,000 copies/µL) to prepare 5 serial dilutions as outlined in Figure 1.
   c. Mix well by vortexing or pipetting after each transfer and before moving to the next transfer.
   d. Add 5 µL of the original supplied concentration as well as the 5 prepared serial dilutions to wells containing the Reaction Master Mix. This will result in 5 concentrations: 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2 and 1×10^1 copies/reaction.
   e. Cap the tubes with the appropriate optical lid and spin briefly at 3000 RPM for a few seconds.

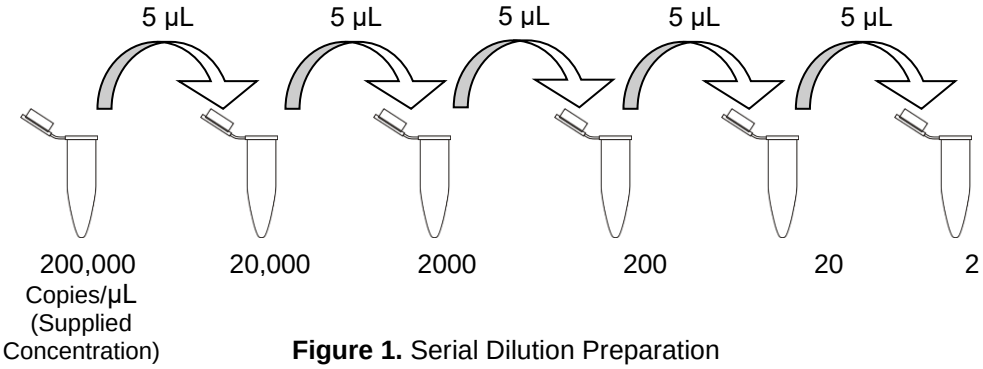

Figure 1. Serial Dilution Preparation

C. Parvo B19 TaqMan PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step PCR.

Table 1. Parvo B19 TaqMan PCR Program.

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

D. Parvo B19 TaqMan PCR Assay Interpretation

i. Qualitative Analysis

Table 2. Interpretation of Assay Results.

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

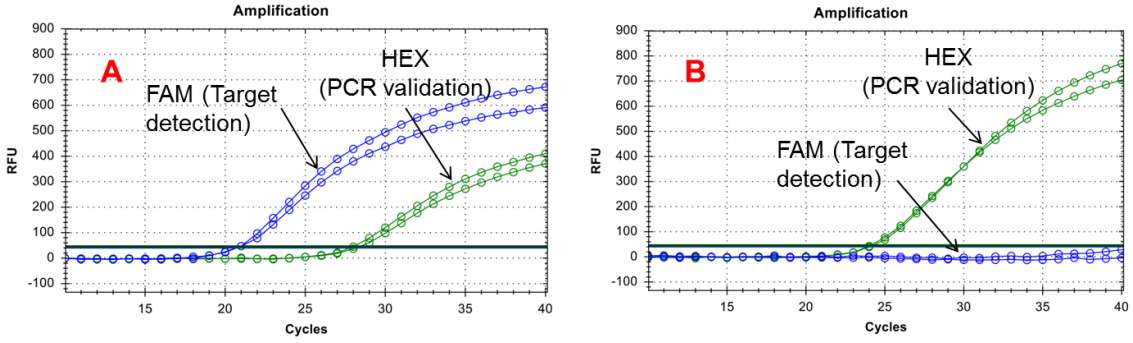

Figure 2. Example of TaqMan PCR positive (A) and negative (B) results. Both PCR signals above the baseline from FAM and HEX channel indicate the successful PCR.

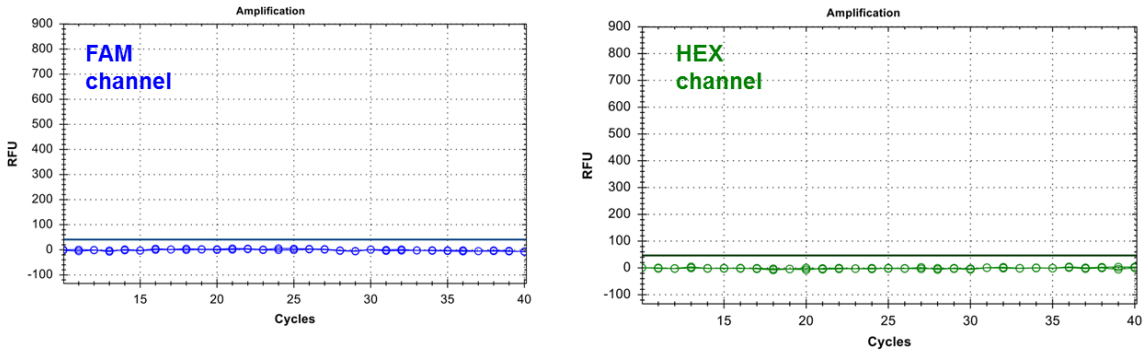

Figure 3. Example of TaqMan PCR inhibition result. No signal from both FAM and HEX channel was detected. It is suggested to repeat the sample preparation using recommended kit for DNA purification.

ii. Quantitative Analysis

1. Use the Ct values obtained from the amplification of the Positive Control and the 5 serial dilutions of the Positive Control to generate a standard curve to illustrate the linear relation between log starting Positive Control copy number (Log Copy Number; X axis) and the obtained Ct value of each concentration (Y axis).
2. Add the linear trend line to the graph and obtain the Correlation Coefficient (R2), slope (m) and intercept (b).
3. Use the slope to calculate the PCR efficiency by the following equation:

   PCR efficiency (%) = ((10^(-1/slope))-1)*100

4. For a valid quantitative diagnostic run the following control parameters of the standard curve must be achieved:

Control Parameter	Value
R^2	> 0.98
Slope	-2.92 to -3.92
PCR Efficiency	80% to 120%
PCR Control Ct	26 to 30.5

5. Use the Ct value of the test sample together with the slope (m) and intercept (b) to calculate the initial test sample concentration from the following equation:

   Initial concentration = $10^{(Ct-b)/m}$

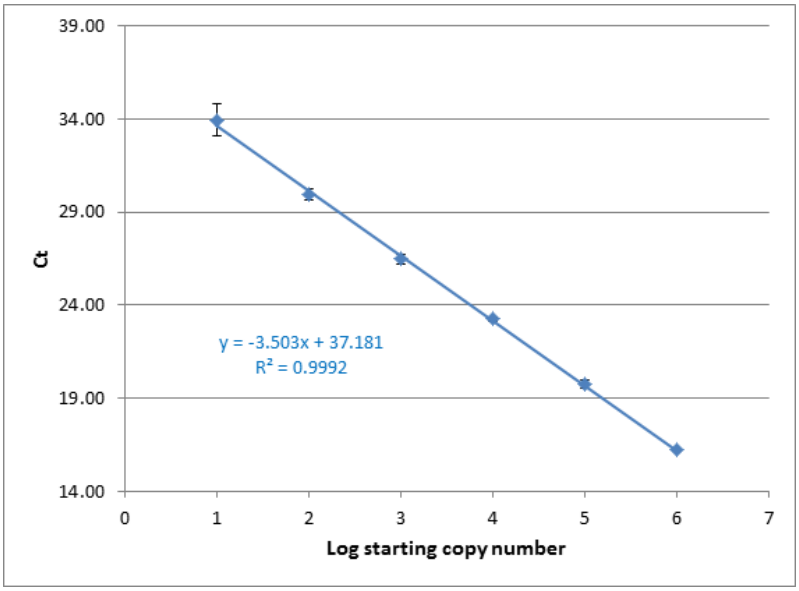

Figure 4. Standard curve between log starting copy number of Parvo B19 Positive Control Standard and Ct value.

E. Performance Evaluation

i. Analytical Sensitivity

The analytical sensitivity of the Parvo B19 TaqMan PCR Kit Dx was determined by analyzing a dilution series of quantified Parvo B19 DNA.

Figure 5. Analytical Sensitivity of the Parvo B19 PCR Kit Dx. A serial dilution (A to F) of Parvo B19 DNA was amplified using Norgen's Parvo B19 TaqMan PCR Kit

Table 3. PCR Results used to determine the Analytical Sensitivity of the Parvo B19 TaqMan PCR Kit Dx.

Standard Sample	Parvo B19 Copy Number Per PCR Reaction (20 µL)	Parvo B19 Detection
A	1.00E+06	Positive
B	1.00E+05	Positive
C	1.00E+04	Positive
D	1.00E+03	Positive
E	1.00E+02	Positive
F	1.00E+01	Positive
NTC	0	Negative

Therefore, the limit of detection (LoD) for Parvo B19 using the Parvo B19 TaqMan PCR Kit Dx is 10 copies.

ii. Analytical Specificity

The analytical specificity of the Parvo B19 TaqMan PCR Kit Dx is ensured by the selection of the Parvo B19 -specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies in published sequences by sequence comparison analysis.

Furthermore, the specificity of the Parvo B19 specific primers were evaluated by testing a panel of DNA extracted from related pathogens.

Figure 6. Analytical Specificity of the Parvo B19 TaqMan PCR Kit Dx. DNA from related pathogens (see Table 2) was amplified using Norgen's Parvo B19 TaqMan PCR Kit. A: detection of Parvo B19, B: PCR internal control.

Table 4. Organisms tested to demonstrate the analytical specificity of the Parvo B19 TaqMan PCR Kit Dx.

Pathogen	Parvo B19 Detection	Internal Control
Parvovirus B19	Positive	Positive
CMV Strain: AD-169	Negative	Positive
EBV	Negative	Positive
HPV	Negative	Positive
VZV	Negative	Positive
HIV	Negative	Positive

The Parvo B19 TaqMan PCR Kit Dx did not cross-react with any of the specified organisms.

iii. Precision

Precision data of the Parvo B19 TaqMan PCR Kit Dx was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Variability data are expressed as a percentage of accurate Parvo B19 detection. The data is based on three different operators performing two experiments each with the same diluted standard (6 dilutions) sample. Each of the 2 experiments carried out by each operator used different production lots. Therefore, 14 replicates per sample were analyzed for intra-assay, inter-assay and inter-lot variability. The precision data was found to be 100% in all cases (intra-assay variability, inter-assay variability, and inter-lot variability).

Table 5. Precision Data for the Parvo B19 TaqMan PCR Kit Dx.

Parvo B19 Precision	R^2	Slope	Efficiency
Intra-Assay Variability	100% PASS	100% PASS	100% PASS
Inter-Assay Variability	100% PASS	100% PASS	100% PASS
Inter-Lot Variability	100% PASS	100% PASS	100% PASS

iv. Reproducibility

Specificity, sensitivity and precision of the Parvo B19 TaqMan PCR Kit Dx were evaluated by carrying out an Internal Split-Sample Proficiency Testing Procedure, in which 2 different Parvo B19 samples were split between 3 operators and analyzed in 3 replicates over 2 different tests.

Product Use Restriction
Norgen's Parvo B19 TaqMan PCR Kit Dx is an *in vitro* diagnostic test for the detection and quantification of Parvo B19 specific DNA using real-time hybridization-fluorescence detection.

Norgen's Parvo B19 TaqMan PCR Kit Dx is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR and in vitro diagnostic procedures.

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 Parvo B19 genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.

As with any diagnostic test, results generated using Norgen's Parvo B19 TaqMan PCR Kit Dx should be interpreted with regard to other clinical or laboratory findings.

The respective user is liable for any and all damages resulting from application of Norgen's Parvo B19 TaqMan PCR Kit Dx 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.

Technical Support
Contact our Technical Support Team between the hours of 9:00 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 support@norgenbiotek.com.

Authorized Representative

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

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