NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

End-Point PCR/RT-PCR Kit

General Product Insert

Product# EPxxxxx

Intended Use

Norgen's End-Point PCR/RT-PCR Kits are designed for the detection of specific pathogen/viral/bacterial DNA or viral/viroid/bacterial RNA based on the use of end-point PCR/RT-PCR technology. These kits are designed for research use only and not for use in diagnostic procedures.

Product Description

The kit is ready-to-use including Master Mix and primers for the amplification of a specific region of the viral/viroid/bacterial 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 specific DNA/RNA is based on end-point PCR/RT-PCR technology, utilizing polymerase chain reaction (PCR) or Reverse transcription polymerase chain reaction (RT-PCR) for the amplification of specific DNA/RNA sequences. For analysis of the data, the PCR/RT-PCR reaction is loaded on an agarose DNA/RNA gel along with the provided DNA ladder for qualitative analysis.

Norgen's End-Point PCR Kit were 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

Component	Product # EPxxxxx (24 preps)
MDx 2X PCR (or RT-PCR) Master Mix	350 µL
Primer Mix	70 µL
Positive Control	50 µL
Nuclease-Free Water	1.25 mL
Loading Dye	100 µL
DNA Ladder	100 µL
Product Insert	1

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 or RNA Purification Kit
    - These kits are compatible with all DNA or RNA purification kits that yield high quality, inhibitor-free DNA or RNA, but were optimized with Norgen Biotek Purification products
- 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 End-Point PCR Kits is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- 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/RT-PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the viral/bacterial/viroid 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/RNA is the starting material for Norgen's End-Point PCR/ RT-PCR Kit. The quality of the DNA/RNA template will have a major impact on the performance of the detection test. The user must ensure that the method used for DNA/RNA purification is compatible with end-point PCR/RT-PCR. We recommend the use of Norgen's RNA and DNA Purification Products which have been fully validated with Norgen's PCR Kits.

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers, 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/RNA. This will help to prevent the carry-over of any ethanol into the purified DNA/RNA, 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/RNA.**

B. PCR/RT-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/RT-PCR Master Mix provided is enough for up to 34 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For every PCR/RT-PCR run, one reaction containing Positive Control and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA/RNA samples tested per PCR run is 6.
- To avoid any contamination while preparing the PCR/RT-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/RT-PCR Negative Control (Table 1)
    2. Prepare the PCR/RT-PCR Pathogen/Viroid/Virus Assay (Table 2)
    3. Prepare the PCR/RT-PCR 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) Master Mix; 3) Primer Mix; and 4) the Sample DNA/RNA or Positive Control).

1. For each PCR/RT-PCR set, prepare **one** no template control PCR as shown in Table 1 below:

Table 1. PCR/RT-PCR Negative Control Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	8 µL
MDx 2X PCR/RT-PCR Master Mix	10 µL
Primer Mix	2 µL
Total Volume	20 µL

2. Prepare the PCR/RT-PCR reaction for sample detection as shown in Table 2 below. The recommended amount of sample DNA/RNA to be used is 2.5 µL. However, a volume

between 1 and 5 µL of sample DNA/RNA may be used as template. Adjust the final volume of the PCR/RT-PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 2. PCR CMV Assay Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	5.5 µL
MDx 2X PCR/ RT-PCR Master Mix	10 µL
Primer Mix	2 µL
Sample DNA	2.5 µL
Total Volume	20 µL

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
MDx 2X PCR/ RT-PCR Master Mix	10 µL
Primer Mix	2 µL
Positive Control (PosC)	8 µL
Total Volume	20 µL

C. PCR/RT-PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. SEE A) for PCR Programming; SEE B) for RT-PCR Programming

a) Run one step PCR

Table 4. Assay Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**3 min**
Cycle 2 (40x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 3	**Step 1**	**72°C**	**5 min**
Cycle 4	**Step 1**	**4°C**	**∞**

b) Run one step RT-PCR.

Table 4. Assay Program

One Step RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**50°C**	**30 min**
Cycle 2	**Step 1**	**95°C**	**3 min**
Cycle 3 (40x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 4	**Step 1**	**72°C**	**5 min**
Cycle 5	**Step 1**	**4°C**	**∞**

D. PCR/RT-PCR Assay Interpretation

- For the analysis of the PCR/RT-PCR data, the entire 20 µL PCR/RT-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/RT-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 PCR Assay Results

Input Type	Target amplification		Interpretation
	Target Band (xxx bp)	**PCR control Band (150 bp)**	
Positive Control	Yes	Yes	Valid
Positive Control	Yes	No	Valid
Negative Control	No	Yes	Valid
Sample	Yes	Yes	Positive
Sample	No	Yes	Negative
Sample	No	No	PCR inhibition
Sample	Yes	No	Positive

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

Examples:

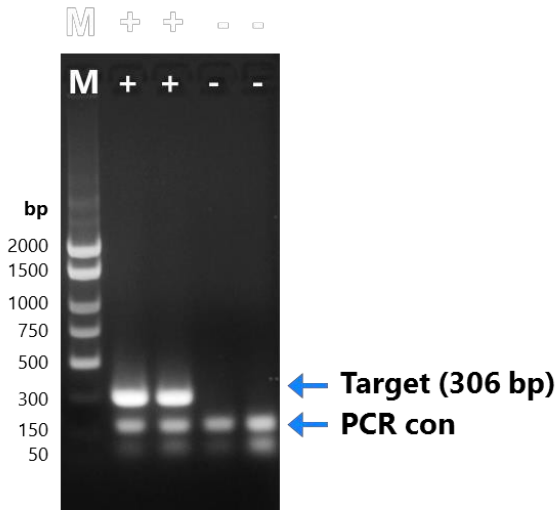

Figure 1: A representative 1X TAE, 1.4 % agarose gel showing the amplification of *CMV* at different concentrations. The size of the *CMV* target amplicon corresponds to the 306 bp band represented by the provided DNA Marker (M).

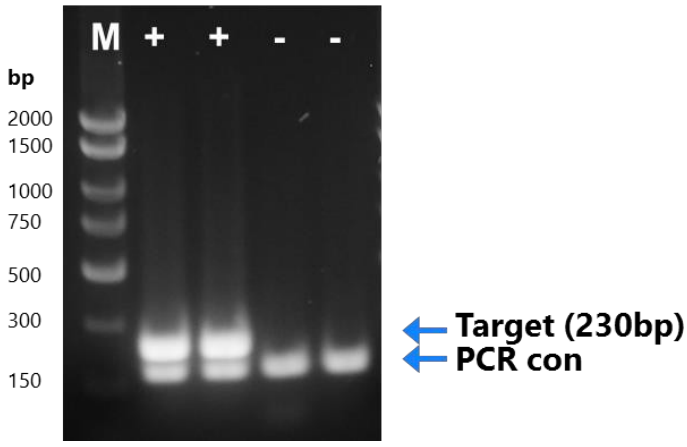

Figure 2: A representative 1X TAE, 1.4 % agarose gel showing the amplification of ASBVd at different concentrations. The size of the ASBVd target amplicon corresponds to the 230 bp band represented by the provided DNA Marker (M).

E. PCR Assay Specificity

The specificity of Norgen's End-Point PCR Kit is first and foremost ensured by the selection of the specific primers, as well as the selection of stringent reaction conditions. The primers were

checked for possible homologies to all human related viruses in GenBank published sequences by sequence comparison analysis.

Frequently Asked Questions

1. How many samples should be included per PCR/RT-PCR run?

- Norgen's 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 PCR/RT-PCR control and the target showed amplification in a sample?

- The assay has to be repeated. It could happen when there are carryover contamination.

3. How should it be interpreted if only the target was amplified in a sample?

- The sample tested should be considered as positive. At high DNA/RNA input, the amplicon will be predominant and thus the PCR control may not amplify as they compete for PCR resources.

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.

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