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TaqMan PCR/RT-PCR Kit — General Product Insert

Product# TMxxxxx

Intended Use

Norgen's TaqMan PCR/RT-PCR Kit is designed for the detection of specific DNA/RNA 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.

Product Description

The detection of specific pathogen/bacteria/viral/viroid DNA/RNA is based on TaqMan PCR/RT-PCR providing a simple, reliable and rapid result. Norgen's TaqMan PCR/RT-PCR Kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The TaqMan PCR/RT-PCR Kit 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 TaqMan PCR/RT-PCR Kit was 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 # TMxxxxx (24 preps)
MDx TaqMan 2X PCR/RT-PCR Master	350 µL
Primer & Probe Mix	70 µL
Positive Control	50 µL
Nuclease-Free Water (Negative control)	1.25 mL
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 Real-Time PCR Instrument with FAM and HEX filter channel
- 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
- PCR reaction preparation station (Optional)

Quality Control

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

Warnings and Precautions

- Norgen's SYBR Green PCR/RT-PCR Kit is intended 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 inhibitors may cause false negative or invalid results.

- Potential mutations within the target regions of the 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 TaqMan Green 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 TaqMan Green 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. TaqMan PCR Assay Preparation

Notes:

- Before use, suitable amounts of all TaqMan PCR/RT-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/RT-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 every TaqMan PCR/ One-Step 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 TaqMan PCR run is 6.
- To avoid any contamination while preparing the TaqMan PCR/One-step 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 *Aspergillus niger* Assay (Table 2)
    3. Prepare the PCR/RT-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) Primer & Probe Mix; 3) Primer Set; and 4) the Sample DNA/RNA or Positive Control).

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

Table 1. TaqMan PCR Negative Control Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix)
Nuclease-Free Water	8 µL
MDx TaqMan 2X PCR /RT-PCR Master Mix	10 µL
Primer & Probe Mix	2 µL
Total Volume	20 µL

2. Prepare the PCR reaction for sample detection as shown in Table 2 below.

Table 2. TaqMan PCR *Aspergillus niger* Assay Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix)
Nuclease-Free Water	5 µL
MDx TaqMan 2X PCR /RT-PCR Master Mix	10 µL
Primer & Probe Mix	2 µL
Sample DNA*	3 µL
Total Volume	20 µL

* The recommended amount of sample DNA/RNA to be used is 3 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 reaction to 20 µL using the Nuclease-Free Water provided.

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

Table 3. TaqMan PCR Positive Control Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix)
MDx TaqMan 2X PCR/RT-PCR Master Mix	10 µL
Primer & Probe Mix	2 µL
Positive Control (PosC)	8 µL
Total Volume	20 µL

C. TaqMan PCR/RT-PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run TaqMan PCR (Table 4) or One-Step RT-PCR (Table 5) assay.

Table 4. *Aspergillus niger* TaqMan PCR Program

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

Table 5. TaqMan One-Step RT-PCR 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	95°C	15 sec
	Step 2	60°C	30 sec

D. TaqMan PCR/RT-PCR Assay Interpretation

Table 6. Interpretation of Assay Results

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

For results obtained that are not covered in Table 6, please refer to the Frequently Asked Questions.

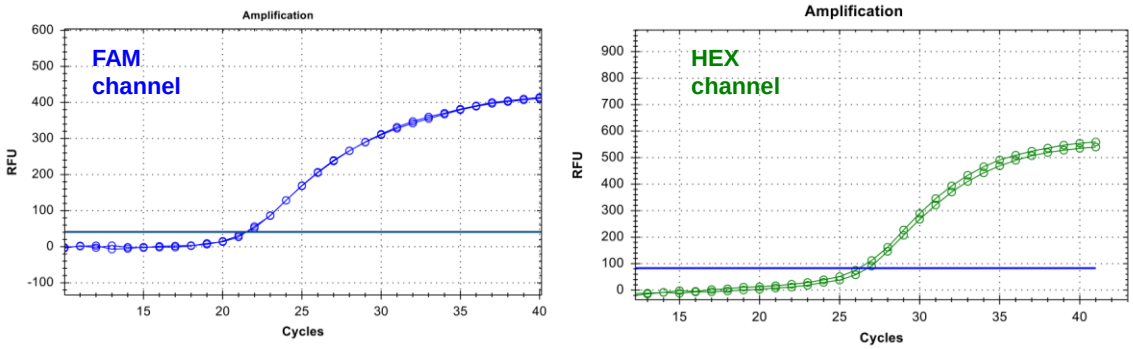

Figure 1. Example of TaqMan PCR Positive result. Both PCR signals above the baseline from FAM and HEX channel indicate the successful PCR.

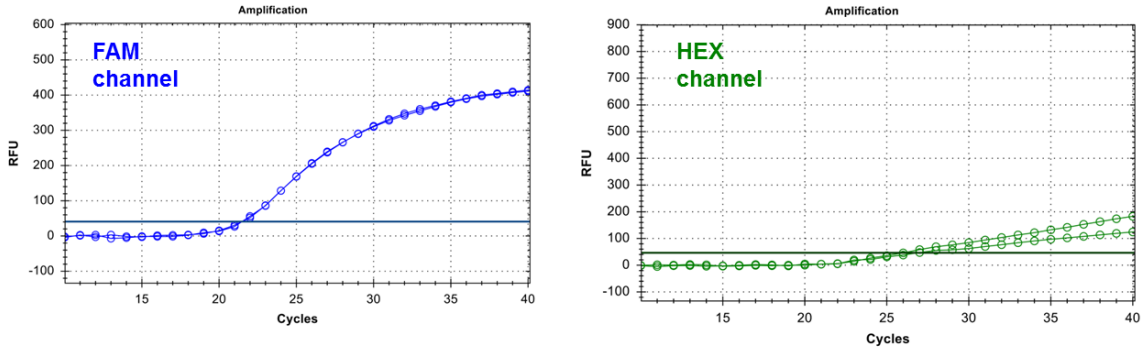

Figure 2. Example of TaqMan One-step RT-PCR Positive result. Both PCR signals above the baseline from FAM and HEX channel indicate the successful PCR.

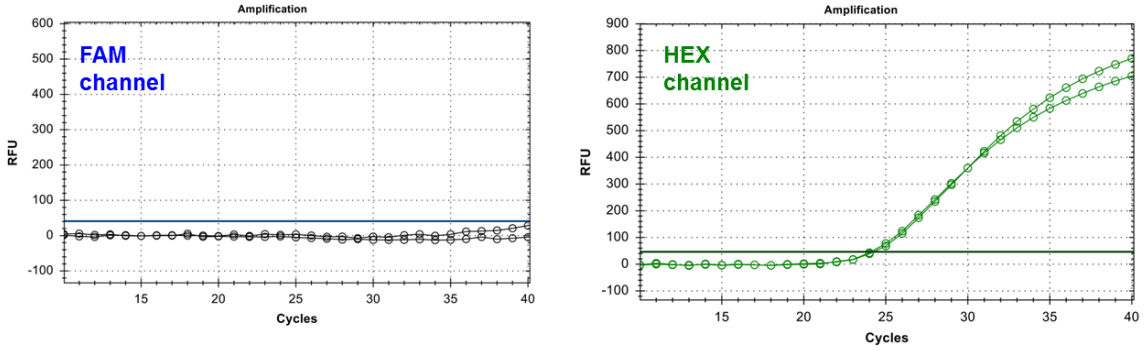

Figure 3. Example of TaqMan PCR Negative result. No target DNA was detected in FAM channel but amplification signal from HEX indicates the successful PCR.

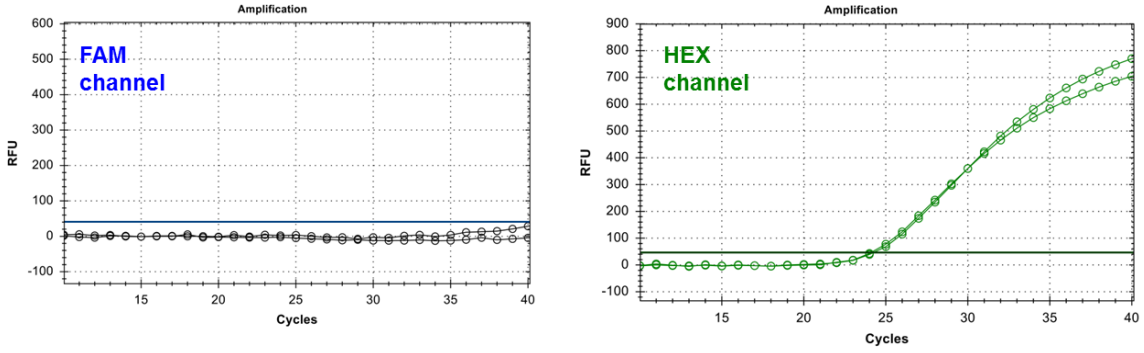

Figure 4. Example of TaqMan One-step RT-PCR Negative result. No target RNA was detected in FAM channel but amplification signal from HEX indicates the successful PCR.

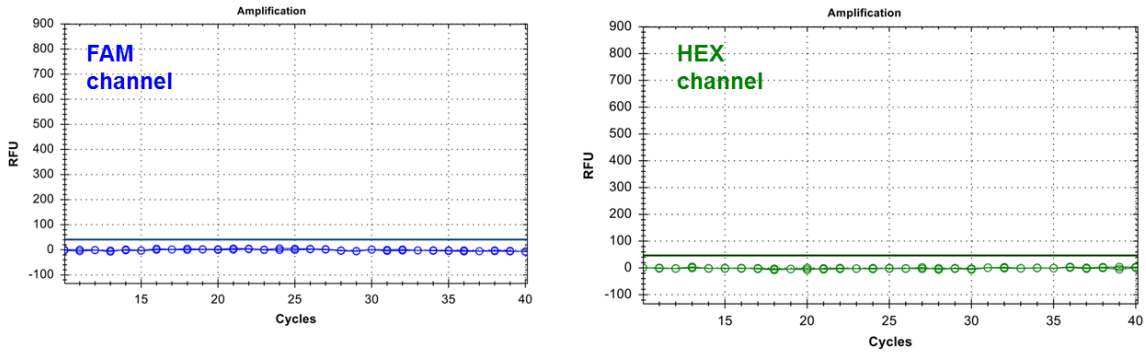

Figure 5. Example of TaqMan PCR inhibition result. No signal from both FAM and HEX channel was detected. It is suggested to repeat the sample preparation using recommended kit for DNA purification.

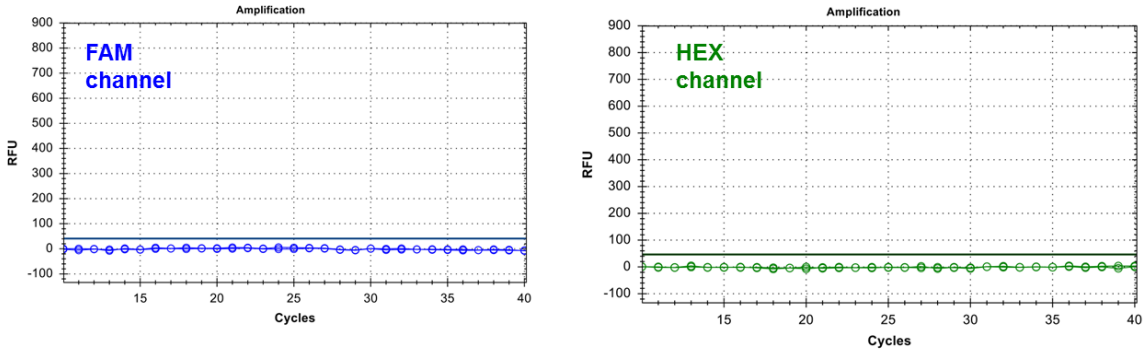

Figure 6. Example of TaqMan one-step RT-PCR inhibition result. No signal from both FAM and HEX channel was detected. It is suggested to repeat the sample preparation using recommended kit for RNA purification.

E. TaqMan PCR/RT-PCR Assay Specificity

The specificity of Norgen's PCR/RT-PCR Detection 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 microorganisms 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 TaqMan PCR/RT-PCR Detection 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 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.

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.

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