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Mastitis pathogens TaqMan PCR Kit Product Insert

Product# TM65950

Intended Use

Norgen's Mastitis pathogens TaqMan PCR Kit is designed for the detection of *Brucella abortus* DNA, *Listeria monocytogenes* DNA, and *Staphylococcus aureus* DNA in a single PCR reaction. This kit is designed for research use only and not for use in diagnostic procedures.

Background Information

Mastitis is an infection in the mammary gland that causes inflammation in the udder or breast tissues. This infection occurs when bacteria invade the teat canal and, as a result, white blood cells are released into the mammary gland (Ibrahim, 2017). Mastitis infection is known for the negative impacts on the quantity and quality of milk produced by dairy animals. It has been shown an estimate reduction of 30 % in milk production per year and a 15 % reduction per cow lactation (Radostits et al., 2007). The main mode of transmission is through contaminated materials, hands, flies found in the milking parlor, contaminated machines, and the consumption of contaminated dairy products (Ibrahim, 2017). There is a wide range of bacterial pathogens that can cause mastitis, some of which include *Brucella abortus, Listeria monocytogenes,* and *Staphylococcus aureus.*

Brucella abortus (*B. abortus)* is a short rod-shaped Gram-negative bacterium that causes brucellosis, a highly infectious disease that can lead to an udder infection (mastitis) in bovine mammals, (Özer, 1999). The disease can be transmitted through the exposure with infected tissues, urine, or blood, but is mainly spread by the consumption of infected dairy products such raw milk and goat cheese.

Listeria monocytogenes (*L. monocytogenes*) is a rod shaped, intracellular, Gram-positive bacteria that causes many health problems such listeriosis, meningitis, gastroenteritis, encephalitis, perinatal infections, and mastitis (Thakur et al., 2018). Due to the intracellular pathogenic characteristic, *L. monocytogenes* is able to infect various host cells, affecting both humans and animals, and it is considered a ubiquitous food borne pathogen capable of surviving high and low temperature, high salt concentration, and pH changes (Addis et al., 2019). The main form of transmission is through the consumption of contaminated food, water and dairy products, mainly due to poor hygiene and milking procedures (Thakur et al., 2018).

Staphylococcus aureus (*S. aureus*) is a non-motile round shaped Gram-positive bacteria involved in a broad range of infections such as osteomyelitis, skin infections, and pneumonia (Le Maréchal et al., 2011). This bacterium is considered one of the common causes of chronic mastitis infection worldwide in both, human and bovine mammals, and due to its pathogenicity, contagiousness, and low cure rates, *S. aureus* is very difficult to eradicate (Rainard et al. 2018). The main form of transmission is through animal to animal during the milking process, milking hands, and flies.

Product Description

The detection of Mastitis pathogens specific DNA is based on TaqMan PCR providing a simple, reliable and rapid result for the detection of *B. abortus*, *L. monocytogenes*, and *S. aureus* infection. Norgen's Mastitis pathogens TaqMan PCR Kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. This kit contains 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.

This 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
- QuantStudio™ 7 Pro Real-Time PCR System

Kit Components

Component	Product # TM65950 (100 preps)
MDx TaqMan 2X PCR Master Mix	2 x 700 µL
Mastitis pathogens Primer & Probe Mix	280 µL
Mastitis pathogens Positive Control	150 µL
Nuclease-Free Water (Negative control)	1.25 mL
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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 kit components can be stored for 2 years after the date of production without showing any reduction in performance.

Customer-Supplied Reagents and Equipment

- Appropriate Real-Time PCR Instrument with FAM, Cy5, Tex615 and HEX filter channels
- DNA Purification Kit
    - The kit is compatible with all DNA purification kits that yield high quality, inhibitor-free DNA
    - **Recommended Purification Kit:** Norgen's Milk Bacterial DNA Isolation Kit (Cat. 21550) or Norgen's Food DNA Isolation Kit (Cat. 54500)
- 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 Mastitis pathogens TaqMan PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Norgen's Mastitis TaqMan 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 2 years.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of any of the target genomes 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 is the starting material for Norgen's Mastitis pathogens TaqMan PCR Kit. The quality of the DNA template will have a major impact on the performance of the target pathogen detection test. The user must ensure that the method used for DNA purification is compatible with TaqMan PCR. We recommend the use of Norgen's **Milk Bacterial DNA Isolation Kit (Cat. 21550) or Norgen's Food DNA Isolation Kit (Cat. 54500).**

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. 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 at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
- Work quickly on ice.
- The amount of MDx TaqMan 2X PCR Master Mix provided is enough for up to 128 PCR reactions (96 sample PCR, 16 positive control PCR and 16 no template control PCR).
- For every TaqMan PCR run, one reaction containing Mastitis pathogens 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 TaqMan PCR run is 6.

- To avoid any contamination while preparing the TaqMan 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 Negative Control (Table 1)
    2. Prepare the PCR Mastitis pathogens Assay (Table 2)
    3. Prepare the 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 or Positive Control).

1. For each TaqMan 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 Master Mix	10 µL
Mastitis pathogens 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 Mastitis pathogens Assay Preparation

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

* The recommended amount of sample DNA to be used is 3 µL. However, a volume between 1 and 5 µL of sample DNA 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 set, prepare **one** positive control 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 Master Mix	10 µL
Mastitis pathogens Primer & Probe Mix	2 µL
Mastitis pathogens Positive Control (PosC)	8 µL
Total Volume	20 µL

C. Mastitis pathogens TaqMan PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 4 below.
2. Run TaqMan PCR assay.

Table 4. Mastitis pathogens TaqMan PCR Program

One Step RT-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. Channel selection for specified platforms.

B. abortus	*L. monocytogenes*	*S. aureus*	Internal Control	Platform
FAM	Texas 615 / *Texas Red	Cy5	HEX	BioRad CFX96 Touch™ Real-Time PCR Detection System,
FAM	JUN/ *ROX	Cy5	VIC	QuantStudio™
Green	Orange	Red	Yellow	Qiagen Rotor-Gene Q

*Note: When referencing two channels, use the first listed channel primarily, and if it not available on the model, use the second.

D. Mastitis pathogens TaqMan PCR Assay Interpretation

Table 6. Interpretation of Assay Results

FAM (*B. abortus* detection)	Tex615 (L. monocytogenes detection)	Cy5 (*S. aureus* detection)	HEX (PCR validation)	Interpretation
+	+	+	+/-	*B. abortus*, *L. monocytogenes*, S. aureus +
+	-	+	+/-	*B. abortus*, *S. aureus* +
+	+	-	+/-	*B. abortus*, *L. monocytogenes* +
+	-	-	+/-	*B. abortus* +
-	+	+	+/-	*L. monocytogenes*, *S. aureus* +
-	-	+	+/-	*S. aureus* +
-	+	-	+/-	*L. monocytogenes* +
-	-	-	+	Negative result
-	-	-	-	Invalid PCR

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

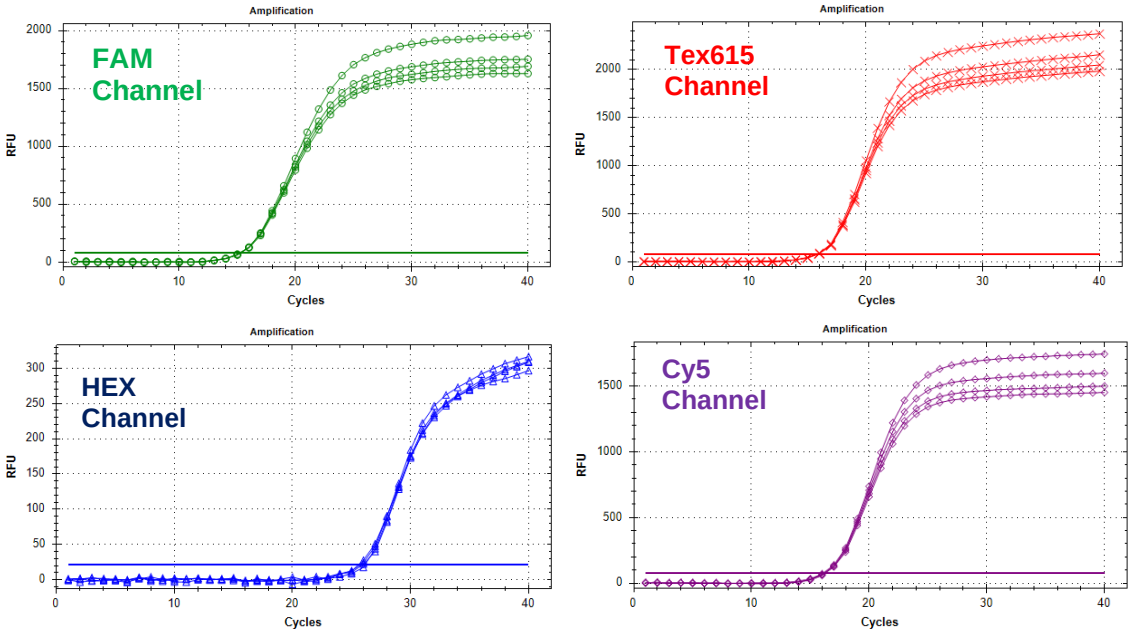

Figure 1. Example of TaqMan PCR Positive result. PCR signals above the baseline from FAM, Tex615, Cy5, and HEX channel indicate the successful PCR. Individual amplification in any of the channels also indicates a positive result for the pathogen corresponding to that channel.

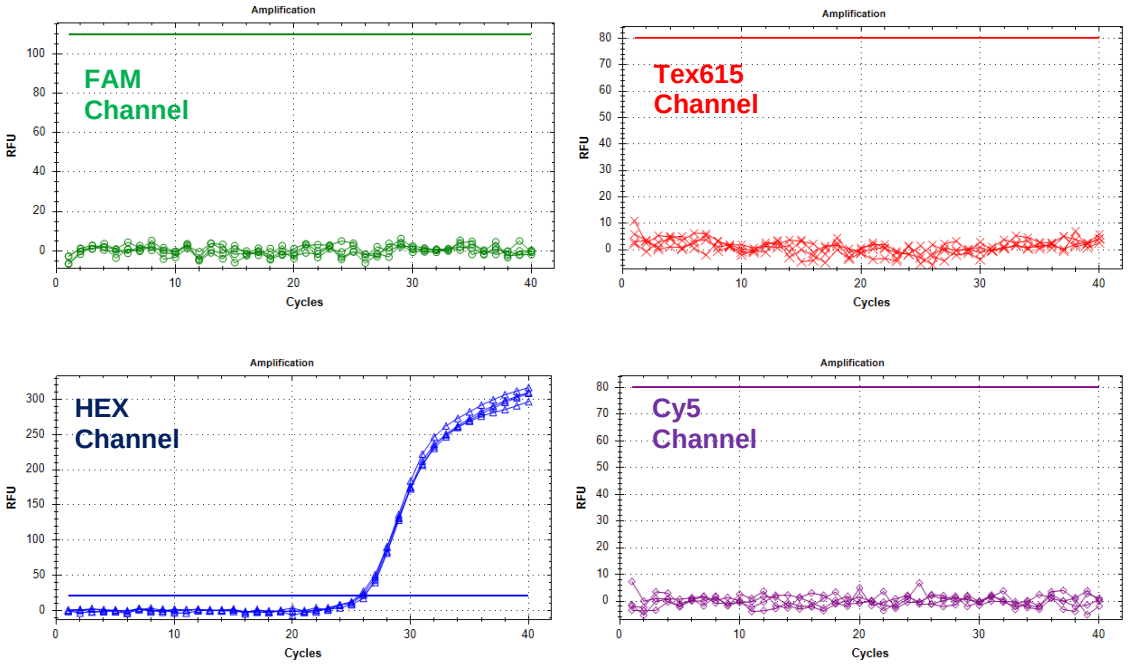

Figure 2. Example of TaqMan PCR Negative result. No target DNA was detected in FAM,TEX615, and Cy5 channels but amplification signal from HEX indicates the successful PCR.

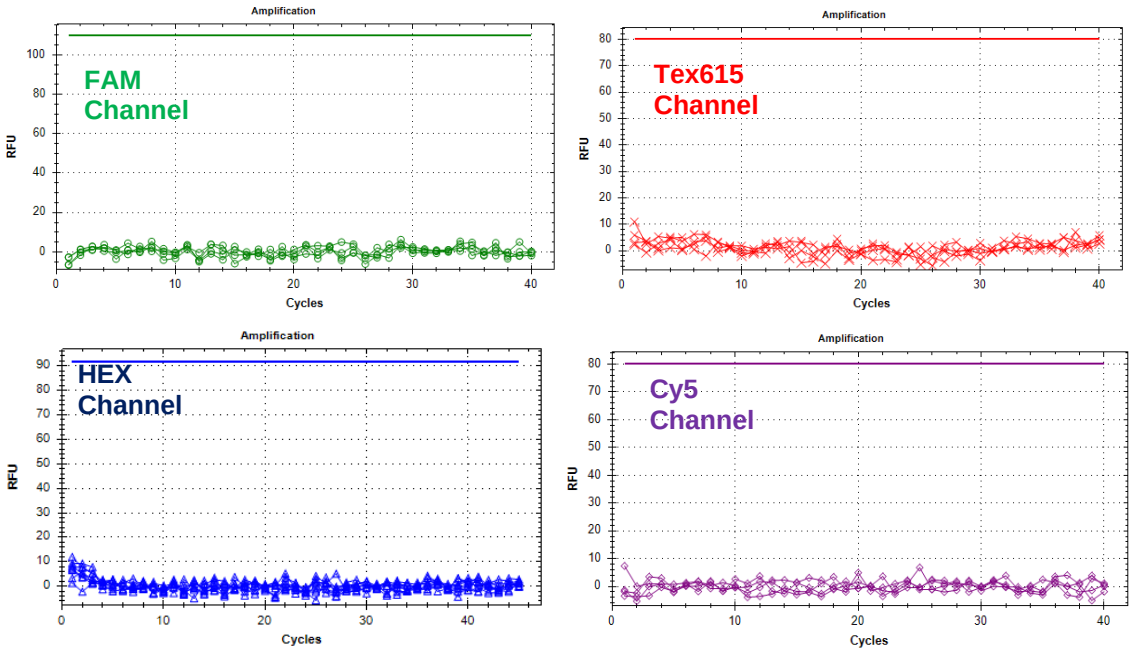

Figure 3. Example of TaqMan PCR inhibition result. No signal from all targets was detected. It is suggested to repeat the sample preparation using recommended kit for DNA purification.

E. Mastitis pathogensTaqMan PCR Assay Specificity

The specificity of Norgen's Mastitis pathogens TaqMan PCR Kit is first and foremost ensured by the selection of the target-specific primers and probes, as well as the selection of stringent reaction conditions. The target primers were checked for possible homologies to all microorganism in GenBank published sequences by sequence comparison analysis.

Frequently Asked Questions

1. **How many samples should be included per PCR run?**
   - This kit is designed to test 96 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.
   - To interpret the results correctly, the positive control should be amplifying at least the FAM, Cy5, and TEX615 channels, and the negative result be amplifying only the HEX channel.

2. **How should it be interpreted if no PCR control signal (HEX) is detected while one or more of the target specific signals (FAM, Cy5, Tex615) is detected in the sample?**
   - Tested samples(s) can be considered positive. The internal control is designed to be a weak signal to ensure it does not compete with the target and lower the sensitivity of the assay.

3. **How should it be interpreted if a target specific signal (FAM, Cy5, Tex615) 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, Cy5, Tex615) is detected in positive control?**
   - It could happen when the positive control was not added. Repeat the assay.

Related Products	Product #
Listeria monocytogenes TaqMan Probe/Primer and Control Set	TM30410
Staphylococcus aureus TaqMan Probe/Primer and Control Set	TM29310
Mastitis TaqMan Probe/Primer and Control Set	TM65910
Milk Bacterial DNA Isolation Kit	21550
Food DNA Isolation Kit	54500

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.

Product Use Restriction

Norgen's Mastitis pathogens TaqMan PCR Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR. This kit is designed for research use only and not for use in 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.

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

TaqMan is a registered trademark of Roche Molecular Systems, Inc

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