



## PathoProof PCR assays

Revolutionary, rapid, and reliable  
identification of mastitis-causing  
microorganisms from bovine milk

# PathoProof PCR assays

PathoProof PCR assays identify mastitis-causing bacteria from bovine milk typically in just four hours. The assays are based on accurate and sensitive real-time PCR. The tests can be used for analyzing fresh and preserved quarter milk samples, milk recording samples, and bulk tank milk samples.

## From sample to results in four hours

PathoProof PCR assays are revolutionary tests for identifying all main mastitis-causing microorganisms from bovine milk samples, without any bacterial culturing steps. The results are obtained typically in just four hours, with only two hours of hands-on time. This is a dramatic improvement over conventional bacterial culturing, which routinely takes 48 hours, and up to ten days for *Mycoplasma* species.

## Analysis from different sample types

The PathoProof PCR assays can be used for milk testing from quarter milk samples and milk recording samples, as well as from bulk tank milk samples. The samples can be preserved (e.g., with bronopol), which minimizes bacterial growth during transportation. This enables shipment of samples at room temperature. The ability to use milk recording samples makes mastitis testing incredibly convenient for dairy producers. Testing bulk tank milk samples is an efficient way to simultaneously screen an entire herd for contagious mastitis-causing bacteria, such as *Streptococcus agalactiae* and *Mycoplasma bovis*.

## Sensitive and accurate results

In addition to identifying viable mastitis bacteria, the PathoProof PCR assays also detect growth-inhibited microorganisms. This is a major advantage compared to the conventional bacterial culture method, where up to 40% of “no growth” results are a problem for laboratories, veterinarians, and dairy producers.

The PathoProof PCR assays simultaneously detect all of the targeted species (see list on last page) present in the milk sample, overcoming the common problem of “mixed growth” results of bacterial culturing. Validation studies indicate superior analytical specificity and sensitivity for the assays, making PCR the most accurate mastitis testing method available.

## Results interpretation and reporting made easy

The PathoProof Software performs automated interpretation and reporting of the results obtained using the assays. Unlike with identification of bacteria from culture plates, all users of the PathoProof PCR assays can now achieve the same very high level of accuracy.

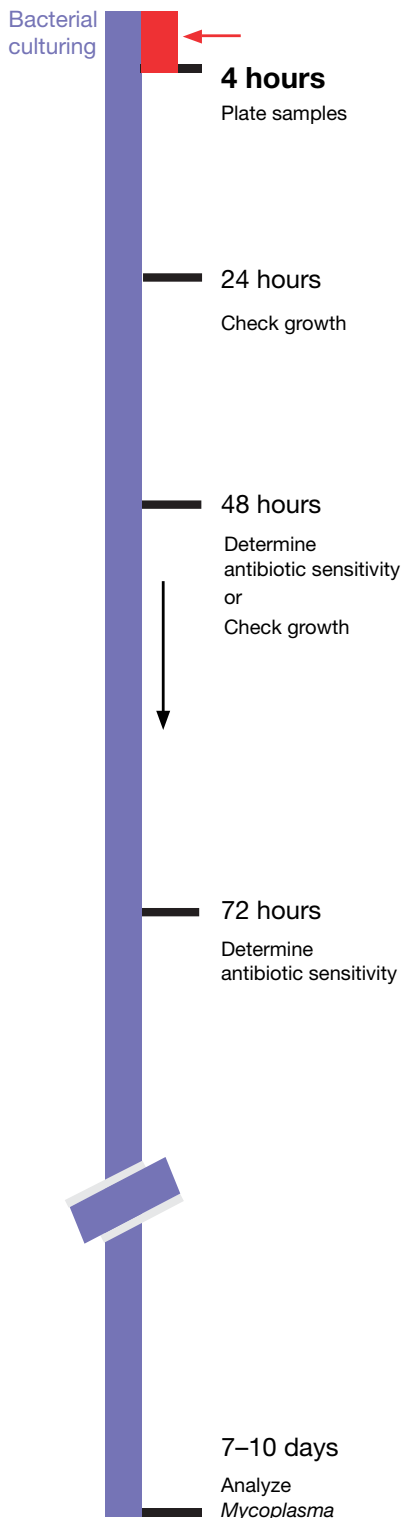
## Maximising milk productivity, safeguarding milk quality and sustaining herd health are important goals of every dairy operation.

Good-quality milk products can only be produced from good-quality raw milk, which must meet accepted standards for chemical composition and purity as well as levels of a variety of mastitis-causing micro-organisms. Additionally the health of the individual cows, barn maintenance, and good hygiene are of utmost importance to minimize the contamination of the milk. Rapid identification of milk contaminated by microorganisms will:

- Ensure that milk and milk products are safe and suitable for their intended uses
- Reduce the time for infective agents to spread to healthy herd mates
- Enable faster decisions on treatment and reduce use of broad spectrum antibiotic treatment
- Remove affected cows from the herd sooner to ensure optimal milk quality

## Results typically in four hours

- All main mastitis-causing microorganisms identified
- *Mycoplasma* identified
- *Staphylococcal* beta-lactamase gene resistance determined



## Three simple steps

### Step 1: Milk sample preparation

DNA of the bacteria present in the milk sample is extracted and purified from all other milk components. The DNA extraction can be done either manually using spin columns or using a semiautomated KingFisher system for magnetic bead-based DNA purification. Using the KingFisher DNA extraction system significantly reduces the hands-on time and time to results when processing larger sample amounts.

### Step 2: Automatic analysis of bacteria

The purified bacterial DNA is amplified to a detectable level using an automated real-time PCR instrument. All mastitis-causing pathogens are identified and semiquantified simultaneously. Loading of the sample plates into the instrument is the only manual step required.

### Step 3: Reporting results

The PathoProof Software is designed for automatically interpreting, reporting, and storing the results.

Polymerase chain reaction (PCR) technology is used for amplifying DNA in a test tube. Amplification enables further analysis of DNA. Millions of copies of any desired DNA fragment can be synthesized in less than one hour. Advanced PCR technology called real-time PCR even allows detection of the amplified DNA simultaneously with the amplification. In the PathoProof PCR assays, several bacterium-specific DNA fragments are amplified from a milk sample in order to determine which mastitis-causing pathogens are present in the sample and how much of each pathogen is present at semiquantitative scale.

# Advantages

- No bacterial culturing required
- Designed to accurately identify all major mastitis-causing pathogens
- From sample to results in typically just four hours
- High sensitivity, even for isolates that show “no growth” in culture
- Suitable for preserved milk samples
- Samples can be shipped at room temperature
- Can be used with milk recording samples
- Tests for both contagious and environmental factors

## **Thermo Scientific™ PathoProof™ Mycoplasma-8**

### **Kit identifies:**

- *Mycoplasma* spp.
- *Mycoplasma alkalescens*
- *Mycoplasma bovis*
- *Mycoplasma bovigenitalium*
- *Mycoplasma canadense*
- *Mycoplasma californicum*
- *Staphylococcus aureus*
- *Streptococcus agalactiae*

## **Thermo Scientific™ PathoProof™ Complete-16** **Kit identifies:**

- *Staphylococcus aureus*
- *Staphylococcus* spp. (including all major coagulase-negative staphylococci)
- *Streptococcus agalactiae*
- *Streptococcus dysgalactiae*
- *Streptococcus uberis*
- *Escherichia coli*
- *Enterococcus* spp. (including *E. faecalis* and *E. faecium*)
- *Klebsiella oxytoca* and/or *K. pneumoniae*
- *Serratia marcescens*
- *Corynebacterium bovis*
- *Trueperella pyogenes* and/or *Peptoniphilus indolicus*
- Staphylococcal  $\beta$ -lactamase gene (penicillin-resistance gene)
- *Mycoplasma bovis*
- *Mycoplasma* spp.
- Yeast
- *Prototheca* spp.

## **Thermo Scientific™ PathoProof™ Major-3** **Kits identify:**

- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Mycoplasma bovis*

## **Thermo Scientific™ PathoProof™ Major-4.2** **Kit identifies:**

- *Mycoplasma bovis*
- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Streptococcus uberis*

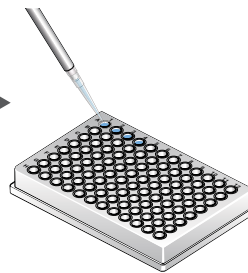
For a full list of available PathoProof assays, see ordering information or go to **[thermoscientific.com](http://thermoscientific.com)**



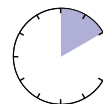




## Workflow



2 hours

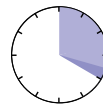


1.5 hours



Sample	<i>S. aureus</i>	<i>S. uberis</i>	<i>E. coli</i>
123	-	+	-
124	+	+	-
125	+	-	-
126	-	+	-
127	-	-	-

30 minutes



Total time required

**4 hours**



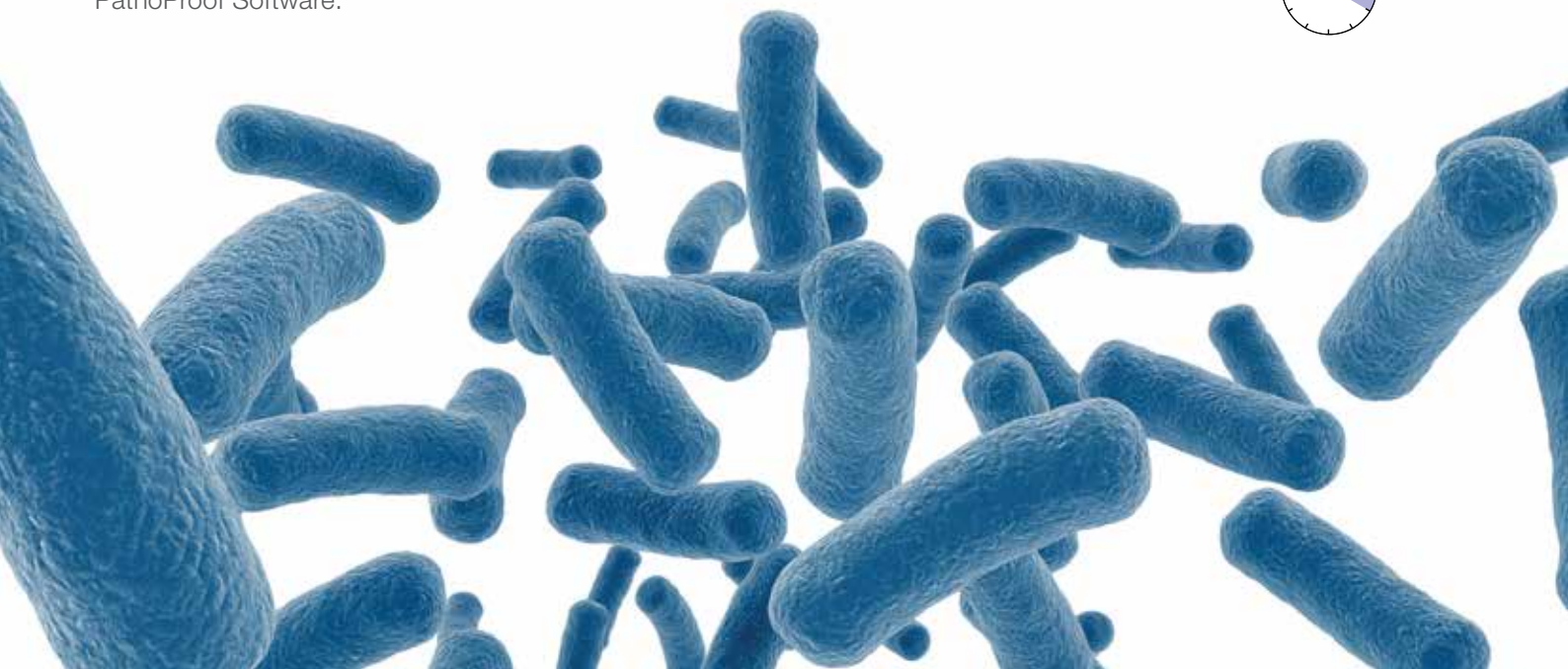
## How to get started

Thermo Scientific™ PathoProof™ PCR assays are based on technology typically applied in molecular biology laboratories. We can assist you in setting up this technology in your laboratory. The following items are needed to get started with PathoProof technology:

- Applied Biosystems™ 7500 or Applied Biosystems™ 7500 Fast Real-time PCR System—an instrument for automatic analysis of microorganisms
- Thermo Scientific™ PathoProof™ Software for analyzing results
- Optional: Thermo Scientific™ KingFisher™ Flex system for automated magnetic bead-based DNA purification

## Comprehensive training included

We provide both the instrument installation and thorough hands-on training for laboratory staff. The training session covers sample preparation, proper use of the real-time PCR instrument, and results interpretation using PathoProof Software.



## Ordering information

Product	No. of tests	Cat. No.
PathoProof Major-3	50	PF3050S
qPCR reagents for use with PF3050S	50	PF3050SB
PathoProof Major-3	384	PF3050L
PathoProof Major-3 KF	384	PF3050LKF
qPCR reagents for use with PF3050L and F3050LKF	384	PF3050LB
PathoProof Major-4.2	50	PF4250S
qPCR reagents for use with PF4250S	50	PF4250SB
PathoProof Major-4.2	384	PF4250L
PathoProof Major-4.2 KF	384	PF4250LKF
qPCR reagents for use with PF4250L and F4250LKF	384	PF4250LB
PathoProof Mycoplasma-8	50	PF8100S
qPCR reagents for use with PF8100S	50	PF8100SB
PathoProof Mycoplasma-8	384	PF8100L
PathoProof Mycoplasma-8 KF	384	PF8100LKF
qPCR reagents for use with PF8100L and PF 100LKF	384	PF8100LB
PathoProof Complete-12	50	PF1250S
qPCR reagents for use with PF1250S	50	PF1250SB
PathoProof Complete-12	384	PF1250L
PathoProof Complete-12 KF	384	PF1250LKF
qPCR reagents for use with PF1250L and F1250LKF	384	PF1250LB
PathoProof Complete-16	50	PF1650S
qPCR reagents for use with PF1650S	50	PF1650SB
PathoProof Complete-16	384	PF1650L
PathoProof Complete-16 KF	384	PF1650LKF
qPCR reagents for use with PF1650L and F1650LKF	384	PF1650LB

Product	Cat. No.
<b>PathoProof Software</b>	
PathoProof Software	PF0888A
<b>Instruments</b>	
7500 Fast Real-Time PCR System with Dell™ Notebook	A30299
KingFisher Flex Magnetic Particle Processor with 96 Deep-Well Head	PF0630LKF

For more information, go to  
[thermoscientific.com/pathoproof](http://thermoscientific.com/pathoproof)

This product is intended to be used for the purpose of detection and/or analysis of microorganisms in milk for quality assurance and quality control purposes (Food Testing Applications), as well as for identification, enumeration, or count of microorganisms in raw material sample, process control sample, or finished product sample of an industrial process for the purpose of detecting the presence, absence or amount either of a contaminant or of an intended component (Industrial Microbiology Applications).  
 © 2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Dell is a trademark of Dell Inc.