VetMAX™ MastiType Multi Kit

Real-time PCR detection of 15 mastitis-causing pathogens and the beta-lactamase gene in four separate PCR reactions

Catalog Number A39227

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Applied Biosystems™ VetMAX™ MastiType Multi Kit enables rapid, accurate detection of mastitis-causing pathogens in bovine milk using real-time PCR amplification of DNA unique to each pathogen.

The VetMAX™ MastiType Multi Kit detects 15 pathogens and the staphylococcal beta-lactamase (penicillin resistance) gene in four separate PCR reactions. The DNA targets include:

- *Corynebacterium bovis*
- *Enterococcus* spp.
- *Escherichia coli*
- *Klebsiella oxytoca* and *Klebsiella pneumoniae*
- *Mycoplasma bovis*
- *Mycoplasma* spp.
- *Prototheca* spp.
- *Serratia marcescens*
- *Staphylococcus aureus*
- Staphylococcal beta-lactamase gene
- *Staphylococcus* spp.
- *Streptococcus agalactiae*
- *Streptococcus dysgalactiae*
- *Streptococcus uberis*
- *Trueperella pyogenes* and *Peptoniphilus indolicus*
- Yeast

The kit contains:

- 1 - MastiType Positive Control—A positive control for the PCR reaction components.
- 2 - MastiType Master Mix—Contains a Hot Start DNA polymerase in an optimized PCR buffer with magnesium and dNTPs.
- 3 - MastiType Multi Primer Mix 1—Includes an Internal Amplification Control (IAC) and primers for *S. aureus*, *Enterococcus* spp., *C. bovis*, and *M. bovis*.
- 4 - MastiType Multi Primer Mix 2—Includes an IAC and primers for the beta-lactamase gene, *E. coli*, *Str. dysgalactiae*, and *Mycoplasma* spp.
- 5 - MastiType Multi Primer Mix 3—Includes an IAC and primers for *Staphylococcus* spp. (including all relevant coagulase-negative staphylococci), *Str. agalactiae*, *Str. uberis*, and *Prototheca* spp.
- 6 - MastiType Multi Primer Mix 4—Includes an IAC and primers for *K. oxytoca* and *K. pneumoniae*, *Ser. marcescens*, *T. pyogenes*/*P. indolicus*, and yeast.

Procedure overview

This document provides guidance for DNA extraction and real-time PCR amplification of DNA from mastitis-causing pathogens in bovine milk samples.

In this procedure, DNA is extracted from fresh, frozen, or preserved milk samples (see Table 2 for recommended products for DNA extraction). Extracted DNA samples are added to a PCR reaction mix in a 96-well plate and results are interpreted, reported, and stored using the Animal Health VeriVet Software available on the Connect cloud-based platform. The VeriVet Software allows you to:

- Create a plate layout
- Generate a template file (EDT file) for import to a real-time PCR instrument
- *(For instruments connected to the Connect platform)* Remotely start and monitor the real-time PCR instrument run
- Analyze the run results to generate molecular and immunodiagnostic testing determinations

For information on instrument compatibility, see "Guidelines for the Animal Health VeriVet Software" on page 2.

Contents and storage

Reagents for 100 real-time PCR tests are supplied.

Table 1 VetMAX™ MastiType Multi Kit (Cat. No. A39227)

Contents	Amount	Storage [1]
1 - MastiType Positive Control[2]	1 × 440 µL	–20°C
2 - MastiType Master Mix	4 × 1200 µL	
3 - MastiType Multi Primer Mix 1[3]	1 × 550 µL	
4 - MastiType Multi Primer Mix 2[3]	1 × 550 µL	
5 - MastiType Multi Primer Mix 3[3]	1 × 550 µL	
6 - MastiType Multi Primer Mix 4[3]	1 × 550 µL	

[1] See packaging for expiration date.

[2] We recommend storing in aliquots (4 tubes of 110 µL), to avoid cross-contamination.

[3] Includes primers and template DNA for an Internal Amplification Control (IAC).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 2 Recommended products for DNA extraction

Item	Source
MagMAX™ CORE Nucleic Acid Purification Kit	A32700
MagMAX™ CORE Mastitis & Panbacteria Module[1]	A39522

[1] Includes high-throughput and manual laboratory protocols.

Table 3 Other required materials

Item	Source
Real-time PCR system, one of the following:	
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System running SDS Software v2.0 or later version	Contact your local sales office.
QuantStudio™ 5 Real-Time PCR System, 0.1-mL or 0.2-mL	
Software	
Animal Health VeriVet Software[1]	**thermofisher.com/connect**
Equipment	
Microcentrifuge	MLS
Pipettes	MLS
Vortex mixer	MLS

Item	Source
Tubes, plates, and other consumables	
Tubes and plates	thermofisher.com/plastics
Ultra Clear qPCR Caps, strips of 8	AB0866
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Nuclease-free Water	AM9938

[1] Connect cloud-based platform account required.

Guidelines for DNA extraction

- We recommend using the MagMAX™ CORE Mastitis & Panbacteria Module (Cat. No. A39522), a supplemental module for use with the MagMAX™ CORE Nucleic Acid Purification Kit (Cat. No. A32700).
- Purified DNA can be stored at 5°C for up to 3 days, or at –20°C for long-term storage.

Guidelines for real-time PCR

- Follow "Good laboratory practices for PCR and RT-PCR" on page 4.
- Real-time PCR instruments must be calibrated with the following dyes:
  - 7500/7500 Fast Instrument—FAM™, Cy5™, Texas Red™, VIC™, and TAMRA™ dyes
  - QuantStudio™ 5 Instrument—FAM™, Cy5™, JUN™, VIC™, and TAMRA™ dyes

  Note: If these dyes are not calibrated on the instrument, contact Technical Support for Spectral Calibration Kit ordering information.
- For each real-time PCR run, include the following control reactions.

Control	Description
Positive Control (PC)	Use 5 µL of the 1 - MastiType Positive Control.
Mastitis Negative Control (MNC)	Use 5 µL of Nuclease-free Water instead of sample DNA.

Guidelines for the Animal Health VeriVet Software

- We recommend using the Animal Health VeriVet Software for the following procedures, according to your instrument type.

Table 4 Compatible instruments and actions

Action	QuantStudio™ 5 Real-Time PCR System	7500/7500 Fast Real-Time PCR Systems
Create a plate layout and generate a template file (EDT file)	✓	—
Remotely start and monitor a run[1]	✓	—
Analyze run results	✓[2]	✓

[1] The instrument must be connected to the Connect cloud-based platform.

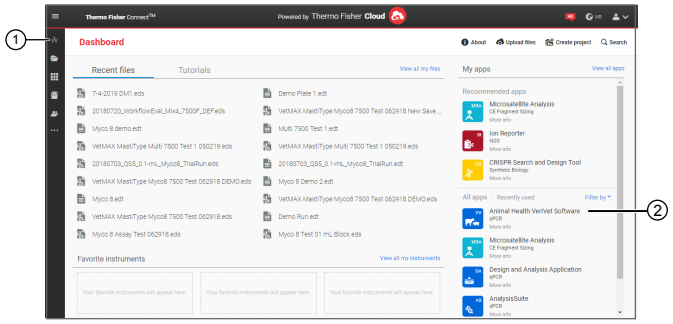
[2] If you import results from a previous run, you must have the QuantStudio™ Design and Analysis SE Software. To download the software, go to **thermofisher.com/quantstudio3-5softwaredownloads**.

- For more information on using the software, see the *Animal Health VeriVet Software Help* available on the Connect cloud-based platform.
- The analysis features of the software require a positive and negative control in each plate.
- For plate setup, assign the following properties to the appropriate wells in the **QUICK SETUP** view:
  - **Assay**—Select **Multi-1**, **Multi-2**, **Multi-3**, or **Multi-4** if you are using the QuantStudio™ 5 Instrument. Select **Multi-1 7500**, **Multi-2 7500**, **Multi-3 7500**, or **Multi-4 7500** if you are using the 7500/7500 Fast Instrument.
  - **Sample**—Enter the unknown sample or control names.
  - **Task**—Assign a Positive Control (**P**) and a Negative Extraction Control/Mastitis Negative Control (**NE**).
  - **Thermal Protocol**—*(QuantStudio™ 5 Instrument only)* The assay-specific thermal protocol is automatically applied.
  - **Block Type**—*(QuantStudio™ 5 Instrument only)* Select the Block Type for your instrument.
- For information on custom calls assigned by the software, see "Interpretation of results" on page 3.

Open the Animal Health VeriVet Software

Sign in to **thermofisher.com/connect** using your Thermo Fisher account.

1. Click in the left sidebar.
2. In the **My apps** pane, click to open the Animal Health VeriVet Software.



① Home icon—Click to return to the **Dashboard**.

② Module icon in the **My apps** pane—Click to open the software.

Before you begin

1. Thaw all frozen reagents on ice, mix by vortexing, then centrifuge the tubes briefly.
2. Thaw the purified sample DNA on ice.

Maintain thawed reagents, controls, and samples at 2–8°C until use.

Prepare the PCR Reaction Mix

Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.

1. Prepare four separate PCR reaction mixes by combining the Master Mix and Primer Mixes in appropriately-sized microcentrifuge tubes according to the following table.

Component	Volume	
	1 well	N[1] wells
PCR Reaction Mix Multi-1		
2 - MastiType Master Mix	10 µL	N × 10 µL
3 - MastiType Multi Primer Mix 1	5 µL	N × 5 µL
PCR Reaction Mix Multi-2		
2 - MastiType Master Mix	10 µL	N × 10 µL
4 - MastiType Multi Primer Mix 2	5 µL	N × 5 µL
PCR Reaction Mix Multi-3		
2 - MastiType Master Mix	10 µL	N × 10 µL
5 - MastiType Multi Primer Mix 3	5 µL	N × 5 µL
PCR Reaction Mix Multi-4		
2 - MastiType Master Mix	10 µL	N × 10 µL
6 - MastiType Multi Primer Mix 4	5 µL	N × 5 µL

[1] N = Number of samples including: Positive Control (**P**), Negative Extraction Control/Mastitis Negative Control (**NE**), and DNA from extracted milk samples.

2. Cap the tubes, then mix the solutions by vortexing.
3. Centrifuge briefly to bring the PCR reaction mixes to the bottom of the tubes and eliminate air bubbles.

Prepare the PCR reaction plate

1. Transfer 15 µL of each PCR reaction mix to the appropriate wells of an optical reaction plate.
2. Add sample or control according to the following table.

Sample type	Component	Volume per reaction
Test sample	Sample DNA	5 µL
Positive Control (**P**)	1 - MastiType Positive Control	5 µL
Negative Extraction Control/Mastitis Negative Control (**NE**)	Nuclease-free Water	5 µL

3. Close the plate with optically clear caps.

   IMPORTANT! Do not use adhesive or heat seals. Assay performance can be adversely affected.

4. Centrifuge briefly to bring the contents to the bottom of the wells and eliminate air bubbles.

Proceed to set up and run the real-time PCR according to your instrument.

- QuantStudio™ 5 Instrument—Use the VeriVet Software to set up the plate layout and run the real-time PCR. See "Guidelines for the Animal Health VeriVet Software" on page 2.
- 7500/7500 Fast Instrument—See "7500/7500 Fast instruments only: Set up, then run the real-time PCR" on page 3.

7500/7500 Fast instruments only: Set up, then run the real-time PCR

For detailed instructions, see the instrument user guide.

1. On the VetMAX™ MastiType Multi Kit product web page (at **thermofisher.com**, search by catalog number), scroll to the **Product Literature** section.
2. Download the appropriate template file (EDT file) for your instrument.
3. Following the manufacturer's instructions, set up the run on your instrument using the following parameters.
   - Reaction volume: 20 µL
   - Run mode: Standard
   - Select the appropriate filter set for the reporter dyes and quenchers.

Target				Reporter	Quencher
Multi-1	**Multi-2**	**Multi-3**	**Multi-4**		
S. aureus	Beta-lactamase gene	*Prototheca* spp.	*Klebsiella* spp.	FAM™ dye	None
C. bovis	*Str. dysgalactiae*	*Str. uberis*	*T. pyogenes*/*P. indolicus*	Cy5™ dye	None
Enterococcus spp.	*E. coli*	*Str. agalactiae*	*Ser. marcescens*	Texas Red™ dye	None
M. bovis	*Mycoplasma* spp.	*Staphylococcus* spp.	Yeast	TAMRA™ dye	None
IAC	IAC	IAC	IAC	VIC™ dye	None

4. Set up the thermal protocol for your instrument.

Stage	Repetitions	Temperature	Time
1	1	95°C	10 minutes
2	40	95°C	5 seconds
		60°C	1 minute

5. Run the thermal cycler program, collecting real-time amplification data during stage 2.
6. *(Recommended)* When the instrument run is complete, transfer the EDS file to a folder that is accessible to the computer running the Animal Health VeriVet Software.

Use the VeriVet Software to import the EDS file and analyze the results. See "Guidelines for the Animal Health VeriVet Software" on page 2.

Validation criteria

Verify that your real-time PCR run is valid before analyzing test sample results.

The test is validated if the following criteria are met.

Reaction type	C_t value for pathogen DNA targets
Positive Control (**P**)	• *Staphylococcus* spp.: <34[1] • Beta-lactamase gene: <36[1] • All other DNA targets: <37[1]
Negative Extraction Control/Mastitis Negative Control (**NE**)	No detection[2]

[1] Samples with a C_t greater than this value are considered suspect due to poor signal-to-noise ratio.

[2] The run is invalid if the C_t value for pathogen DNA is <38 for the NE. If the C_t value is 38–40, the PCR may be contaminated. See "Troubleshooting" on page 4.

Assay	C_t value for IAC[1]
Multi-1	20–33
Multi-2	20–29
Multi-3	20–26
Multi-4	16–22

[1] If the C_t value is outside of the indicated range, see "Troubleshooting" on page 4.

Interpretation of results

Table 5 Interpretation of sample results

Target result	Custom call	Interpretation
Negative	—	Pathogen DNA is not detected.
Positive	+	Pathogen DNA is detected in low quantity.
Positive	++	Pathogen DNA is detected in intermediate quantity.
Positive	+++	Pathogen DNA is detected in high quantity.
Suspect	—	Pathogen DNA is detected in quantity above the assay's cut-off value.
Inconclusive	—	IAC failed and pathogen DNA is not detected.

Note: C_t values >37 but <40 can indicate that the target is present at low levels. We recommend that C_t values >37 are considered negative.

Troubleshooting

Observation	Possible cause	Recommended action
All test samples and negative control: The C_t values of the Internal Amplification Control (IAC) are not within the acceptable range in the samples and in the negative control wells.	Reagents are missing in the PCR setup.	Repeat the real-time PCR with fresh reagents.
	The wrong volume of Master Mix and/or Primer Mix was used.	Repeat the real-time PCR. Ensure that the correct amounts of Master Mix and Primer Mix are added to the correct wells.
All test samples: Unacceptable IAC amplification signals for all samples. Acceptable IAC signals for the negative control wells.	PCR inhibitors from the DNA extraction are present in the samples.	See *MagMAX™ CORE Mastitis & Panbacteria Module User Guide* (Pub. No. MAN0017800).
Test sample: Unacceptable IAC amplification signals for all replicate reactions for one sample. Acceptable IAC signals in other samples and in the negative control wells.	The PCR inhibitor concentration in the sample is too high.	Dilute the DNA sample 1:5 or 1:10, then repeat the real-time PCR with the diluted DNA.
Negative control: Unacceptable IAC amplification signals for one reaction or all replicate reactions for the negative control. Acceptable IAC signals for sample wells.	Incorrect volume of reagents in the negative control.	No action required because the IAC signals in the samples are acceptable.
Negative control: Positive pathogen target amplification signals in negative control wells.	Carryover contamination.	See "Good laboratory practices for PCR and RT-PCR" on page 4.
Amplification curve: The amplification curve is not smooth and/or is linear.	An adhesive seal or heat seal was used to close the PCR plate. The heating protocol that is used in VetMAX™ MastiType kits can stretch the seal on wells, resulting in abnormal signal reads.	Use Ultra Clear qPCR Caps to avoid false-positive signal reads.

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision history: Pub. No. MAN0017654

Revision	Date	Description
B.0	7 October 2019	Updated to reflect changes to the Animal Health VeriVet Software user interface. Removed the QuantStudio™ 5 Real-Time PCR System from the plate setup and run topic. Minor formatting changes.
A.0	18 July 2018	New document.