MagMAX™ CORE Mastitis & Panbacteria Module

USER GUIDE

Automated and manual purification of high-quality nucleic acid from all bacteria, including species known to cause mastitis in cattle

for use with:
MagMAX™ CORE Nucleic Acid Purification Kit
KingFisher™ Flex Purification System

Catalog Number A39522
Publication Number MAN0017800
Revision A.0

For Laboratory Use Including Veterinary and Environmental Uses.

ThermoFisher SCIENTIFIC

Manufacturer: Thermo Fisher Scientific | 7 Kingsland Grange | Warrington, Cheshire WA1 4SR | United Kingdom | Made in Finland.

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Revision	Date	Description
A.0	20 June 2018	New document.

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Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The MagMAX™ CORE Mastitis & Panbacteria Module is a supplemental module for use with the MagMAX™ CORE Nucleic Acid Purification Kit (Cat. No. A32700). It is optimized for processing fresh, frozen, or preserved milk samples for the detection of pathogens that are known to cause mastitis in cattle, including gram-positive and gram-negative bacteria. The module uses magnetic bead-based separation, and it is compatible with both automated (using the KingFisher™ Flex Purification System) and manual purification methods.

Contents and storage

Table 1 MagMAX™ CORE Mastitis & Panbacteria Module (Cat. No. A39522)

Contents	Amount	Storage
MagMAX™ CORE Mastitis Panbacteria solution	4 × 1375 µL	–20°C[1]

[1] This product is stable for one year when stored as indicated.

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 MagMAX™ CORE Nucleic Acid Purification Kit

Contents	Cat. No. A32700 (100 reactions)	Cat. No. A32702 (500 reactions)	Storage
MagMAX™ CORE Lysis Solution	50 mL	275 mL	15–30°C (room temperature)
MagMAX™ CORE Binding Solution	45 mL	220 mL	
MagMAX™ CORE Wash Solution 1	60 mL	300 mL	
MagMAX™ CORE Wash Solution 2	60 mL	300 mL	
MagMAX™ CORE Elution Buffer	12 mL	55 mL	
MagMAX™ CORE Magnetic Beads	2.2 mL	11 mL	
MagMAX™ CORE Proteinase K (20 mg/mL)	1.25 mL	5 mL	

Table 3 Materials required for the MagMAX™ CORE Nucleic Acid Purification Kit

Item	Source
Instrument and equipment	
KingFisher™ Flex Purification System See page 14 for materials required for the manual purification method.	Contact your local sales office.
Laboratory mixer, Vortex or equivalent	MLS
Tubes, plates, and other consumables	
Adhesive PCR Plate Foils, or equivalent	AB0626
KingFisher™ Flex Microtiter Deepwell 96 plates, 50 plates	95040460
KingFisher™ 96 KF microplates (200 µL), 48 plates	97002540
KingFisher™ 96 tip comb for DW magnets, 100 combs	97002534
Reagents	
(*Optional*) Internal positive control (IPC), one of the following: [1]	
VetMAX™ Xeno™ Internal Positive Control DNA	A29764
VetMAX™ Xeno™ Internal Positive Control RNA	A29763
IPC supplied with your VetMAX™ PCR Kit	**thermofisher.com**

[1] Not required for VetMAX™ MastiType kits.

Table 4 Materials required for the MagMAX™ CORE Mastitis & Panbacteria Module

Item	Source
Boekel Scientific™ 270300 Microplate Shaker, or equivalent	Fisher Scientific™ 15-600-325
(Optional) Magnetic-Ring Stand (96 well)	AM10050

Workflow

Set up the processing plates (page 10) ▼ Prepare the Lysis/Binding/Bead Mix (page 10)	MagMAX™ CORE Nucleic Acid Purification Kit
▼ Prepare the sample (page 11) ▼ Combine the sample with MagMAX™ CORE Mastitis Panbacteria solution, then add Proteinase K (page 11)	MagMAX™ CORE Mastitis & Panbacteria Module
▼ Process samples on the instrument and add the Lysis/Binding/Bead Mix (page 12)	MagMAX™ CORE Nucleic Acid Purification Kit

2 Before you begin

Procedural guidelines

- Before use, invert bottles of solutions and buffers to ensure thorough mixing.
- Mix samples with reagents by pipetting up and down.
- To prevent cross-contamination:
  - Cover the plate or tube strip during the incubation and shaking steps, to prevent spill-over.
  - Carefully pipet reagents and samples, to avoid splashing.
- To prevent nuclease contamination:
  - Wear laboratory gloves during the procedures. Gloves protect you from the reagents, and they protect the nucleic acid from nucleases that are present on skin.
  - Use nucleic acid-free pipette tips to handle the reagents, and avoid putting used tips into the reagent containers.
  - Decontaminate lab benches and pipettes before you begin.

Before first use of the kit

Determine the maximum plate shaker setting

If a plate shaker is used, determine the maximum setting.

1. Verify that the plate fits securely on your shaker.
2. Add 1 mL of water to each well of the plate, then cover with sealing foil.
3. Determine the maximum setting that you can use on your shaker without any of the water splashing onto the sealing foil.

Download and install the script

The appropriate script for the MagMAX™ CORE Nucleic Acid Purification Kit must be installed on the instrument before first use.

1. On the MagMAX™ CORE Nucleic Acid Purification Kit product web page (at **thermofisher.com**, search by catalogue number), scroll to the **Product Literature** section.

2. Right-click the appropriate file to download the latest version of the MagMAX_CORE script for your instrument.

   Table 5 Recommended scripts

Instrument	Script name
KingFisher™ Flex	MagMAX_CORE_Mastitis.bdz

3. See your instrument user guide or contact Technical Support for instructions for installing the script.

3 Methods

- Follow this procedure for automated purification using the KingFisher™ Flex instrument.
- For manual purification, see Appendix B, "Manual purification method".

Set up the processing plates

1. Set up the processing plates.

Table 6 Plate setup: KingFisher™ Flex instrument

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	MagMAX™ CORE Wash Solution 1	500 µL
Wash Plate 2	3	Deep Well	MagMAX™ CORE Wash Solution 2	500 µL
Elution	4	Standard	MagMAX™ CORE Elution Buffer	90 µL
Tip Comb	5	Standard	Place a tip comb in the plate.	

[1] Position on the instrument.

2. (*Optional*) To prevent evaporation and contamination, cover the prepared processing plates with sealing foil until they are loaded into the instrument.

Prepare the Lysis/Binding/Bead Mix

1. Combine the following components, in the order indicated, for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Lysis Solution	350 µL
MagMAX™ CORE Binding Solution	350 µL
MagMAX™ CORE Magnetic Beads	20 µL
Total Lysis/Binding/Bead Mix (–IPC)	**720 µL**
(*Optional*) Internal positive control (IPC), one of the following:[1]	
VetMAX™ Xeno™ Internal Positive Control DNA	2 µL[2]
VetMAX™ Xeno™ Internal Positive Control RNA	2 µL[2]

Component	Volume per sample
Internal positive control (IPC) supplied with your VetMAX™ PCR Kit	As indicated in the instructions for the kit
Total Lysis/Binding/Bead Mix (+IPC)	**720 µL + volume of IPC**

[1] Not required for VetMAX™ MastiType kits.

[2] Different assays may require different volumes of internal positive control (IPC). See your assay guidelines for IPC recommendations.

2. Vortex the mixture thoroughly.

(*Optional*) Store the Lysis/Binding/Bead Mix at room temperature for up to 24 hours.

Prepare the sample

Prepare the samples and controls as described.

Sample type	Action
Milk	Proceed with 200 µL of fresh, frozen, or preserved milk sample.
Negative Extraction Control[1]	Proceed with 200 µL of Nuclease-free Water or 1X PBS.

[1] Recommended to detect reagent contamination during sample preparation.

Combine the sample with MagMAX™ CORE Mastitis Panbacteria solution, then add Proteinase K

1. Add 50 µL of MagMAX™ CORE Mastitis Panbacteria solution to the required wells in the plate.
2. Add 200 µL of milk sample.
3. Mix the sample with MagMAX™ CORE Mastitis Panbacteria solution for 5 minutes at room temperature according to your mixing method.
   - **Using a plate shaker**—Shake vigorously for 5 minutes (see "Determine the maximum plate shaker setting" on page 8).
   - **By pipetting**—Pipet up and down several times, then incubate for 5 minutes at room temperature.
4. Add 10 µL of MagMAX™ CORE Proteinase K to the required wells in the plate.
5. Immediately proceed to process samples on the instrument (next section).

Process samples on the instrument and add the Lysis/Binding/Bead Mix

1. Select the appropriate script on the instrument (see "Download and install the script" on page 9).
2. Start the run, then load the prepared plates in the appropriate positions when prompted by the instrument.
   The run proceeds for 10 minutes, followed by a pause.
3. While the instrument is paused, remove the sample plate from the instrument.
4. Vortex the tube of Lysis/Binding/Bead Mix to resuspend the beads, then add 720 µL of the Lysis/Binding/Bead Mix to each well.
5. Load the sample plate back on the instrument, then press **Start** to continue the run.
6. When the run is complete, remove the elution plate containing the purified nucleic acid, then cover the plate with adhesive film.

   Note: In samples with high DNA content, beads can carry over into the final eluate. If this occurs, place the elution plate on a magnetic ring stand to allow the beads to settle (~1 minute) before using the eluate for PCR amplification.

Store the purified nucleic acid on ice for immediate use, at −20°C for up to 1 month, or at −80°C for long-term storage.

A

Troubleshooting

Observation	Possible cause	Recommended action
The eluate is light brown in color	Magnetic beads were carried over into the eluate.	A small quantity of beads in the sample does not inhibit PCR reactions. Remove the beads from the eluted nucleic acid by placing the plate or tube strip on a magnetic stand (~1 minute), then transfer the nucleic acid solution to a new nuclease-free plate or tube strip.
Poor or no DNA signal (that is, the C_t value is higher than expected) In test samples, the C_t value of the Internal Amplification Control (IAC) target is outside of the validated value range (non-compliant IAC C_t value; invalid sample).	Inhibitors are present in the recovered nucleic acid. These workflows yield high-quality nucleic acid for most samples. However, samples that contain exceptionally high amounts of inhibitors can carry over inhibitors at levels sufficient to affect PCR.	1. Dilute the invalid nucleic acid sample 1:10 in 1X TE buffer. 2. Perform a new PCR analysis with the diluted nucleic acid. • If the diluted nucleic acid is positive for the target, or if it is negative for the target with a compliant IAC C_t value, the result is validated. • If the diluted nucleic acid is negative for the target with a non-compliant IAC C_t value, the result is not validated. In this case, dilute the original biological sample 1:10 in 1X PBS, then repeat the purification and PCR. If the result is still not validated, then repeat the purification and PCR on a new biological sample.
	Samples with high amounts of nucleic acid can saturate the magnetic beads. Bead saturation reduces nucleic acid recovery.	Dilute the samples 1:2, 1:4, 1:8, and 1:16 in 1X PBS, then repeat the purification and PCR.
Well-to-well variation in DNA yield from replicate samples	The magnetic beads were not fully resuspended/dispersed.	In general, the magnetic beads disperse more easily when the temperature of the mixture is > 20°C. Be sure that you: • Vortex the magnetic beads thoroughly before preparing a bead mix. • Fully resuspend the bead mix before adding it to the samples.
Positive samples are clustered in the PCR plate	High-titer samples (exhibiting a low or early C_t) have contaminated nearby wells.	Repeat the nucleic acid purification of the positive or suspect samples without the high-titer samples.

B Manual purification method

This appendix provides manual purification procedures for the low-throughput processing of nucleic acid from milk samples.

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 7 Materials required for the manual purification method

Item	Source
Benchtop microcentrifuge capable of 15,000 × *g*	MLS
Laboratory mixer, Vortex or equivalent	MLS
Heat block set to 95°C	MLS
DynaMag™–2 Magnet, or equivalent 16-tube magnetic stand	12321D
Vortex Adapter-60, or equivalent	AM10014
Eppendorf™ Snap-Cap Microcentrifuge Safe-Lock™ Tubes, or equivalent 1.5 mL tubes	Fisher Scientific™ 05-402-25

Manual purification procedure

Prepare the Lysis/Binding/ Bead Mix

1. Combine the following components, in the order indicated, for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Lysis Solution	350 µL
MagMAX™ CORE Binding Solution	350 µL
MagMAX™ CORE Magnetic Beads	20 µL
Total Lysis/Binding/Bead Mix (–IPC)	**720 µL**
(*Optional*) Internal positive control (IPC), one of the following:[1]	
VetMAX™ Xeno™ Internal Positive Control DNA	2 µL[2]
VetMAX™ Xeno™ Internal Positive Control RNA	2 µL[2]
Internal positive control (IPC) supplied with your VetMAX™ PCR Kit	As indicated in the instructions for the kit
Total Lysis/Binding/Bead Mix (+IPC)	**720 µL + volume of IPC**

[1] Not required for VetMAX™ MastiType kits.

[2] Different assays may require different volumes of internal positive control (IPC). See your assay guidelines for IPC recommendations.

2. Vortex the mixture thoroughly.

(*Optional*) Store the Lysis/Binding/Bead Mix at room temperature for up to 24 hours.

Prepare the sample

Prepare the samples and controls as described.

Sample type	Action
Milk	Proceed with 200 µL of fresh, frozen, or preserved milk sample.
Negative Extraction Control[1]	Proceed with 200 µL of Nuclease-free Water or 1X PBS.

[1] Recommended to detect reagent contamination during sample preparation.

Combine the sample with MagMAX™ CORE Mastitis Panbacteria solution, then add Proteinase K

1. Add 50 µL of MagMAX™ CORE Mastitis Panbacteria solution to each 1.5-mL tube.
2. Add 200 µL of milk sample.
3. Using a vortex with a microtube adapter, shake at moderate speed for 5 minutes.
4. Centrifuge at 500 × *g* for 10 seconds to bring the contents to the bottom of the tube.
5. Add 10 µL of MagMAX™ CORE Proteinase K to each tube, then shake at moderate speed for 5 minutes.

6. Incubate at 95°C for 5 minutes.
7. Centrifuge at 500 × *g* for 10 seconds to collect condensation that can accumulate in the cap of the tube.

Combine the sample mixture with the Lysis/Binding/ Bead Mix

Note: A 16-tube magnetic stand is required for the remainder of the manual purification procedure.

1. Add 720 µL of the Lysis/Binding/Bead Mix to each tube, then vortex at moderate-to-high speed for 3 minutes.
2. Centrifuge at 500 × *g* for 10 seconds.
3. Place the tubes and tube rack on the magnetic stand, then incubate at room temperature for 3 minutes.
4. With the tubes on the magnetic stand, carefully remove, then discard the supernatant.
5. Remove the tube rack from the magnetic stand.

Wash the sample

1. Add 500 µL of MagMAX™ CORE Wash Solution 1 to each tube.
   a. Vortex at moderate high speed for 1 minute.
   b. Centrifuge at 500 × *g* for 10 seconds.
   c. Place the tubes and tube rack on the magnetic stand, then incubate at room temperature for 1 minute.
   d. With the tubes on the magnetic stand, carefully remove, then discard the supernatant.
   e. Remove the tube rack from the magnetic stand.
2. Add 500 µL of MagMAX™ CORE Wash Solution 2 to each tube.
   a. Vortex at moderate high speed for 1 minute.
   b. Centrifuge at 500 × *g* for 10 seconds.
   c. Place the tubes and tube rack on the magnetic stand, then incubate at room temperature for 1 minute.
   d. With the tubes on the magnetic stand, carefully remove, then discard the supernatant.

Dry the beads

1. Keep the tubes on the magnetic stand and with the tubes open, dry the beads at room temperature for 5 minutes.
2. Use a 20 µL pipette to remove any remaining wash solution.

Elute the nucleic acid

1. Remove the tube rack from the magnetic stand.
2. Add 90 µL of MagMAX™ CORE Elution Buffer to each tube.
3. Vortex at moderate high speed for 3 minutes.
4. Centrifuge at 500 × *g* for 10 seconds.
5. Place the tubes and tube rack on the magnetic stand, then incubate at room temperature for 3 minutes.
6. With the tubes on the magnetic stand, transfer the purified nucleic acid to a clean, labeled tube or plate.

Store the purified nucleic acid on ice for immediate use, at −20°C for up to 1 month, or at −80°C for long-term storage.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
MagMAX™ CORE Nucleic Acid Purification Kit User Guide	MAN0015944
Thermo Scientific™ KingFisher™ Flex User Manual	N07669

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
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  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - 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

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