MagMAX™ CORE Mechanical Lysis Module

USER GUIDE

Automated purification of high-quality nucleic acid from *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and other difficult-to-lyse samples

for use with:
MagMAX™ CORE Nucleic Acid Purification Kit
KingFisher™ Flex Purification System
MagMAX™ Express-96 Deep Well Magnetic Particle Processor
KingFisher™ Duo Prime Purification System
KingFisher™ mL Purification System

Catalog Numbers A32836, A37489, A37487, A37488
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For Laboratory Use Including Veterinary and Environmental Uses.

ThermoFisher SCIENTIFIC

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Revision	Date	Description
A	16 November 2017	New document

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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 Mechanical Lysis Module is a supplemental module for use with the MagMAX™ CORE Nucleic Acid Purification Kit (Cat. Nos. A32700 and A32702). The module is designed for processing tough-to-lyse bacteria in complex sample matrices such as feces. For example, DNA from *Mycobacterium avium* subspecies *paratuberculosis* (MAP), known to cause Johne's Disease in cattle, can be isolated from bovine feces using the procedures that are described in this guide.

The MagMAX™ CORE Nucleic Acid Purification Kit uses a simple magnetic separation process in preparation for downstream molecular analysis, and has been optimized for use with KingFisher™ instruments.

Contents and storage

Table 1 MagMAX™ CORE Mechanical Lysis Module (Cat. No. A32836)

Contents	Amount[1]	Storage
MagMAX™ CORE Clarifying Solution	45 mL	15–30°C
MagMAX™ CORE Bead Beating Tubes	100 tubes (2 mL)	

[1] Also available in combination with the MagMAX™ CORE Nucleic Acid Purification Kit (Cat. No. A37487)

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)[1]	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	

[1] Also available in combination with the MagMAX™ CORE Mechanical Lysis Module (Cat. No. A37487)

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

Item	Source
Instrument and equipment	
One of the following instruments: • KingFisher™ Flex Purification System • MagMAX™ Express-96 Deep Well Magnetic Particle Processor See page 18 for other compatible instruments.	Contact your local sales office.
Benchtop microcentrifuge capable of 15,000 × *g*	MLS
Laboratory mixer, Vortex or equivalent	MLS
Reagents	
PBS, pH 7.4[1]	10010023
(*Optional*) Internal positive control (IPC), one of the following:	
VetMAX™ Xeno™ Internal Positive Control DNA	A29764
VetMAX™ Xeno™ Internal Positive Control RNA	A29763
IPC supplied with your VetMAX™ PCR Kit	thermofisher.com
Tubes, plates, and other consumables	
Adhesive PCR Plate Foils, or equivalent	AB0626
KingFisher™ Flex Microtiter Deepwell 96 plates, 50 plates	95040460

Item	Source
KingFisher™ 96 KF microplates (200 µL), 48 plates	97002540
KingFisher™ 96 tip comb for DW magnets, 100 combs	97002534

[1] Can be used for isolation of host nucleic acid, or when processing easy-to-lyse bacteria.

Table 4 Materials required for the MagMAX™ CORE Mechanical Lysis Module

Item	Source
Instrument and equipment	
Bead mill homogenizer for bead-beating, one of the following, or equivalent: • Fisher Scientific™ Bead Mill 24 Homogenizer (recommended) • Mixer Mill 400 (Verder 207450001) • Precellys™ 24 Homogenizer (Bertin) • FastPrep-24™ Instrument (MP Biomedical 116004500) • Mini-BeadBeater-96 (Glen Mills)	 • Fisher Scientific™ 15-340-163 • Fisher Scientific™ 08 418 241 • Bertin EQ03119.200.RD000.0 • Fisher Scientific™ MP116004500 • Fisher Scientific™ NC0141170
Reagents	
Nuclease-Free Water (or any deionized water)	AM9938

Table 5 Additional materials for the High-input workflow

Item	Source
50-mL conical tube	MLS
(*Optional*) Fisherbrand™ Disposable Sterile Spoon	Fisher Scientific™ 14-375-255
(*Optional*) MagMAX™ CORE Glass Microbeads[1,2]	A37489

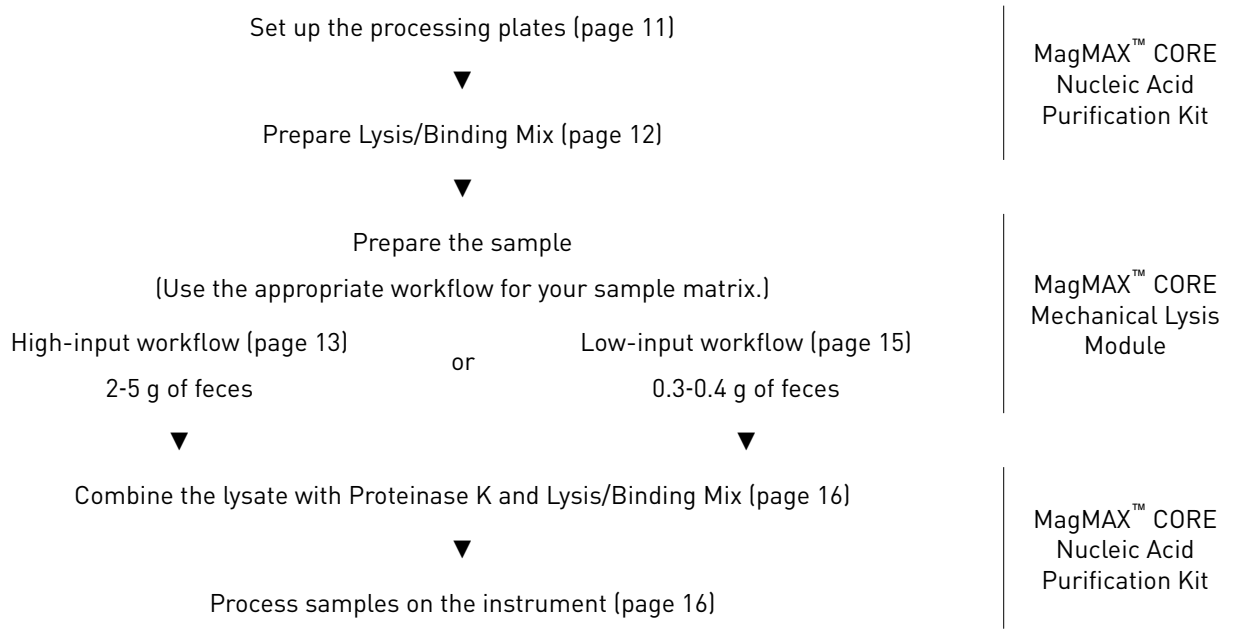
[1] Also available in combination with the MagMAX™ CORE Nucleic Acid Purification Kit and the MagMAX™ CORE Mechanical Lysis Module (Cat. No. A37488)

[2] Required for non-bovine or dried fecal samples.

Table 6 Additional materials for the Low-input workflow

Item	Source
2-mL tube	MLS

Workflow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MagMAX™ CORE Nucleic Acid Purification Kit
Prepare the sample
(Use the appropriate workflow for your sample matrix.)
High-input workflow (page 13)
2-5 g of feces
or
Low-input workflow (page 15)
0.3-0.4 g of feces
MagMAX™ CORE Mechanical Lysis Module
Combine the lysate with Proteinase K and Lysis/Binding Mix (page 16)
Process samples on the instrument (page 16)
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

(*Optional*) Determine the optimal bead mill homogenizer settings

We recommend using the Fisher Scientific™ Bead Mill 24 Homogenizer for maximum nucleic acid yield. If an alternative instrument is used, follow the manufacturer's guidelines to determine the speed and time settings necessary to achieve sufficient cell lysis.

Download the script

The scripts for the MagMAX™ CORE Nucleic Acid Purification Kit are not pre-installed on the instruments.

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 7 Recommended scripts

Instrument	Script name
KingFisher™ Flex	MagMAX_CORE_Flex.bdz
KingFisher™ 96 MagMAX™ Express-96	MagMAX_CORE_KF-96.bdz
KingFisher™ Duo Prime	MagMAX_CORE_DUO.bdz
KingFisher™ mL	MagMAX_CORE_mL_no_heat.bdz

If required by your laboratory, use one of the following scripts, which do not heat the samples during the elution step.

Table 8 Alternate scripts without heated elution step

Instrument	Script name
KingFisher™ Flex	MagMAX_CORE_Flex_no_heat.bdz
KingFisher™ 96 MagMAX™ Express-96	MagMAX_CORE_KF-96_no_heat.bdz
KingFisher™ Duo Prime	MagMAX_CORE_DUO_no_heat.bdz
KingFisher™ mL	MagMAX_CORE_mL_no_heat.bdz

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

3 Methods

Follow this procedure if you are using these instruments:

- KingFisher™ Flex
- MagMAX™ Express-96

Follow Appendix B, "Purification with the KingFisher™ Duo Prime or KingFisher™ mL instrument" if you are using these instruments:

- KingFisher™ Duo Prime
- KingFisher™ mL

Set up the processing plates

1. Set up the processing plates.

Table 9 Plate setup: KingFisher™ Flex or MagMAX™ Express-96 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 Lysis/Binding Mix

1. Combine the following components 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 Mix (–IPC)	**720 µL**
(*Optional*) Internal positive control (IPC), one of the following:	
VetMAX™ Xeno™ Internal Positive Control DNA	2 µL[1]
VetMAX™ Xeno™ Internal Positive Control RNA	2 µL[1]
Internal positive control (IPC) supplied with your VetMAX™ PCR Kit	As indicated in the instructions for the kit
Total Lysis/Binding Mix (+IPC)	**720 µL + volume of IPC**

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

2. Mix by inverting the tube or bottle at least 10 times.

3. Store Lysis/Binding Mix at room temperature for up to 24 hours. Do not store on ice.

Prepare the sample

Select a workflow for your sample matrix.

Option	Description
2-5 g of feces	High-input workflow
0.3-0.4 g of feces	Low-input workflow

High-input workflow

1. Add the following components to a 50-mL conical tube:

Component	Amount
Feces	2-5 g[1]
Deionized water[2]	30 mL
(*Optional*) MagMAX™ CORE Glass Microbeads[3]	3 beads

[1] Equivalent to one scoop, using a Disposable Sterile Spoon (Fisher Scientific 14-375-255).
[2] 1X PBS can be used for isolation of host nucleic acid, or when processing easy-to-lyse bacteria.
[3] Recommended when working with non-bovine or dried fecal samples.

2. Vortex vigorously for 30 seconds, or until the sample is suspended.

3. Incubate at room temperature for 10±2 minutes.

   Note: Incubate samples on a surface that is free of vibration, to allow the contents to settle before transferring the supernatant.

4. Transfer 1.8 mL of supernatant to a 2-mL MagMAX™ CORE Bead Beating Tube.

   Note: If samples are difficult to pipette, the incubation time in step 3 may be increased in 5-minute increments.

5. Centrifuge at 15,000 × *g* for 10 minutes.

6. Remove the supernatant without disturbing the pellet.

   Note: Keep the tube vertical while pipetting to help prevent dislodging the pellet. It is acceptable to aspirate some debris while removing the supernatant.

7. Add 400 µL of MagMAX™ CORE Clarifying Solution to each sample.

8. Disrupt (bead-beat) the samples.

Option	Settings
Fisher Scientific™ Bead Mill 24 Homogenizer	6 m/s; 3 minutes
Mixer Mill 400	30 Hz; 10 minutes
Precellys™ 24 Homogenizer	6,800 rpm; 2 × 90 seconds
FastPrep-24™ Instrument	6.5 M/s; 4 × 45 seconds
Mini-BeadBeater-96	5 minutes

Note: If an alternative bead mill homogenizer is used, see "(Optional) Determine the optimal bead mill homogenizer settings" on page 9.

9. Centrifuge at 15,000 × *g* for 3 minutes.

10. Proceed with 300 µL of supernatant (clarified lysate) to "Combine the lysate with Proteinase K and Lysis/Binding Mix" on page 16.

Store the clarified lysate at room temperature. Recentrifuge the tubes if not used for ≥1 hour.

Low-input workflow

1. Add the following to a new 2-mL tube:

Component	Amount
Feces	0.3-0.4 g
Deionized water[1]	1 mL

[1] 1X PBS can be used for isolation of host nucleic acid, or when processing easy-to-lyse bacteria.

2. Vortex vigorously for 3 minutes, or until the sample is suspended.

3. Centrifuge at 100 × *g* for 30 seconds.

IMPORTANT! Do not centrifuge at higher speeds or for longer than 1 minute. Excessive centrifugation can result in bacteria in the pellet instead of in the fecal supernatant.

4. Add 400 µL of MagMAX™ CORE Clarifying Solution to the required number of MagMAX™ CORE Bead Beating Tubes.

5. Add 175 µL of the fecal supernatant to the MagMAX™ CORE Bead Beating Tube containing 400 µL of MagMAX™ CORE Clarifying Solution.

6. Disrupt (bead-beat) the samples.

Option	Settings
Fisher Scientific™ Bead Mill 24 Homogenizer	6 m/s; 3 minutes
Mixer Mill 400	30 Hz; 10 minutes
Precellys™ 24 Homogenizer	6,800 rpm; 2 × 90 seconds
FastPrep-24™ Instrument	6.5 M/s; 4 × 45 seconds
Mini-BeadBeater-96	5 minutes

Note: If an alternative bead mill homogenizer is used, see "(Optional) Determine the optimal bead mill homogenizer settings" on page 9.

7. Centrifuge at 15,000 × *g* for 3 minutes.

8. Proceed with 300 µL of supernatant (clarified lysate) to "Combine the lysate with Proteinase K and Lysis/Binding Mix" on page 16.

Store the clarified lysate at room temperature. Recentrifuge the tubes if not used for ≥1 hour.

Combine the lysate with Proteinase K and Lysis/Binding Mix

1. Add 10 µL of MagMAX™ CORE Proteinase K to the required wells in the plate or tube strip.
2. Add 300 µL of clarified lysate.
3. Mix the clarified lysate with Proteinase K by pipetting up and down several times, then incubate for 2 minutes at room temperature.
4. Add 720 µL of Lysis/Binding Mix to each sample.
5. Immediately proceed to process samples on the instrument (next section).

Process samples on the instrument

1. Select the appropriate script on the instrument (see "Download the script" on page 10).
2. Start the run, then load the prepared plates or tube strips in their positions when prompted by the instrument.

Store 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
Poor or no RNA or DNA signal (that is, the C_t value is higher than expected)	There are inhibitors in the recovered nucleic acid. These workflows yield high-quality nucleic acid from most samples. However, samples that contain exceptionally high amounts of inhibitors can carry over inhibitors at levels sufficient to affect RT-PCR or PCR.	Include a negative extraction control, to determine the C_t value of the IPC without inhibitors. Reduce the amount of RNA used in the RT-PCR or the amount of DNA used in the PCR: Dilute the eluted nucleic acid 10-fold and repeat the RT-PCR or PCR. If a signal is observed using the diluted sample, inhibitors might be present in the eluted nucleic acid.
	Samples with high amounts of nucleic acid, such as tissue, avian blood, and bacterial cultures, can overwhelm the magnetic beads. Overwhelming the beads reduces nucleic acid extraction efficiency.	Dilute the sample before use, or use less sample.
	Insufficient lysis resulting in low yields.	Increase the speed and time of the bead mill homogenizer during the bead-beating step.
Well-to-well variation in RNA/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.
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 RT-PCR or 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.

B

Purification with the KingFisher™ Duo Prime or KingFisher™ mL instrument

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 10 Materials required for processing on the KingFisher™ Duo Prime instrument

Item	Source
KingFisher™ Duo Prime Purification System	5400110
KingFisher™ Duo Combi pack for Microtiter 96 Deepwell plate (tip combs, plates and elution strips for 96 samples)	97003530
KingFisher™ Duo Elution Strip, 40 pieces[1]	97003520
KingFisher™ Duo 12-tip comb, for Microtiter 96 Deepwell plate, 50 pieces[1]	97003500
KingFisher™ Flex Microtiter Deepwell 96 plates[1]	95040460

[1] Included in the KingFisher™ Duo Combi pack (Cat. No. 97003530).

Table 11 Materials required for processing on the KingFisher™ mL instrument

Item	Source
KingFisher™ mL Purification System	5400050
KingFisher™ mL Tubes and tip combs for 240 samples	97002141
KingFisher™ mL Tip comb, 800 pieces	97002111
KingFisher™ mL Tube, 20 x 45 pieces	97002121

Purification procedure

Note: When performing this procedure for processing on the KingFisher™ mL instrument, mix samples by pipetting up and down. Do not use a plate shaker with the large tube strips required by this instrument.

1. Follow the workflow for your sample type, starting with sample lysate preparation through combining the samples with beads and lysis solution.

   Note: Do not set up processing plates or tubes before preparing samples.

2. Add MagMAX™ CORE Wash Solutions and MagMAX™ CORE Elution Buffer to the indicated positions, according to your instrument.

   Load the Tip Comb and all of the plates or tube strips at the same time. The instrument does not prompt you to load items individually.

Table 12 Plate setup: KingFisher™ Duo Prime instrument

Row ID	Row in the plate	Plate type	Reagent	Volume per well
Sample	A	Deep Well	Sample lysate/bead mix	Varies by sample
Wash 1	B		MagMAX™ CORE Wash Solution 1	500 µL
Wash 2	C		MagMAX™ CORE Wash Solution 2	500 µL
Elution[1]	Separate tube strip[2]	Elution strip	MagMAX™ CORE Elution Buffer	90 µL
Tip Comb	H	Deep Well	Place a tip comb in the plate.	

[1] Ensure that the elution strip is placed in the correct direction in the elution block.

[2] Placed on the heating element.

Table 13 Tube strip setup: KingFisher™ mL instrument

Position ID	Tube strip position	Tube	Reagent	Volume per well
Sample	1	Standard	Sample lysate/bead mix	Varies by sample
Wash 1	2		MagMAX™ CORE Wash Solution 1	500 µL
Wash 2	3		MagMAX™ CORE Wash Solution 2	500 µL
Elution	4		MagMAX™ CORE Elution Buffer	90 µL
Tip Comb	N/A	N/A	Slide the tip comb into the tip comb holder.	

3. Follow "Process samples on the instrument" on page 16.

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
Thermo Scientific™ KingFisher™ Duo Prime Technical Manual	N16621
Thermo Scientific™ KingFisher™ mL User Manual	1508260
Applied Biosystems™ MagMAX™ Express 96 User Manual	N07849

Customer and technical support

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- 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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