MagMAX™ CORE Nucleic Acid Purification Kit

USER GUIDE

Automated purification of high-quality DNA and RNA from veterinary samples

for use with:
KingFisher™ Flex Magnetic Particle Processor
MagMAX™ Express-96 Deep Well Magnetic Particle Processor
KingFisher™ Duo Prime Magnetic Particle Processor
KingFisher™ mL Magnetic Particle Processor

Catalog Numbers A32700, A32702
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For Veterinary Use Only.

ThermoFisher SCIENTIFIC

Manufacturer: Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744

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Revision	Date	Description
B.0	30 June 2017	• Combined and renamed workflows. – Simple: formerly Workflows A and C – Complex: formerly Workflow B – Digestion: formerly Workflow D • Added new workflow: Lysis Incubation. • Added plate processing of samples in the Digestion workflow. • Added list of instrument scripts. • Reorganized into chapters for better navigation and clarity.
A.0	22 December 2016	New document.

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 Nucleic Acid Purification Kit is designed for rapid purification of high-quality DNA and RNA for downstream molecular analysis. The kit uses magnetic bead-based separation, and it is compatible with the following instruments:

- KingFisher™ Flex Magnetic Particle Processor
- MagMAX™ Express-96 Deep Well Magnetic Particle Processor
- KingFisher™ Duo Prime Magnetic Particle Processor
- KingFisher™ mL Magnetic Particle Processor

The kit is optimized for a wide range of sample types. See "Recommended workflows" on page 7.

Contents and storage

Table 1 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	1.25 mL	5 mL	

Required materials not supplied

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

Item	Source
Instrument and equipment	
One of the following instruments: • KingFisher™ Flex Magnetic Particle Processor • MagMAX™ Express-96 Deep Well Magnetic Particle Processor See page 35 for other compatible instruments.	Contact your local sales office.
Benchtop microcentrifuge capable of 15,000 × *g*	MLS
Benchtop centrifuge with plate and tube adaptors	MLS
Laboratory mixer, Vortex or equivalent	MLS
Biotang Inc Microplate Shaker, or equivalent titer plate shaker	Fisher Scientific™ 50-751-4965
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	
Thermo Scientific™ Easy-Peel Heat Sealing Foil, or equivalent	AB-0745
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
Additional materials required for tissue samples in the Simple workflow	
Fisher Scientific™ Fisher Scientific™ Bead Mill 24 Homogenizer	Fisher Scientific™ 15-340-163
PYREX™ Solid Glass Beads for Distillation Columns (3 mm)	Fisher Scientific™ 11-312-10A
Additional materials required for the Digestion workflow	
PK Buffer for MagMAX™-96 DNA Multi-Sample Kit	4489111

[1] Not required for Lysis Incubation workflow.

Recommended workflows

Note: These workflows are not recommended for purification of DNA from tough-to-lyse bacteria, for example, *M. paratuberculosis* (MAP).

Sample matrix	Nucleic acid	Recommended workflow
• Ear punch (circular shape, 2- to 3-mm diameter) in Lysis Solution	Viral nucleic acid	Lysis Incubation[1] (page 29)
• Ear punch (circular shape, 2- to 3-mm diameter) in PBS • Ear notch (triangular shape, approximately 1-cm width) • Milk • Plasma • Serum	• Viral nucleic acid • Bacterial DNA	Simple (page 10)
• Biomed Diagnostics InPouch™ TF (*Tritrichomonas foetus*) culture	*Tritrichomonas foetus* DNA	
• Semen	Viral nucleic acid	
• Swabs—animal • Whole blood	• Viral nucleic acid • Genomic DNA	
• Tissue or organ	• Viral nucleic acid • Bacterial DNA[2]	
	• Bacterial DNA • Genomic DNA	Digestion (page 21)
• Hair follicles	Genomic DNA	
• Environmental samples • Feces • Swabs—environmental or fecal	Bacterial DNA	
	• Viral nucleic acid • Bacterial DNA[2]	Complex (page 15)
• Oral fluid	• Viral nucleic acid • Bacterial DNA	

[1] Recommended if overnight incubation is required.

[2] If concurrent isolation of viral nucleic acid and bacterial DNA is not required, use the Digestion workflow.

2 Before you begin

Procedural guidelines

- Before use, invert bottles of solutions and buffers to ensure thorough mixing.
- Mix samples with reagents using a plate shaker or by pipetting up and down.

  Note: Do not use a plate shaker with the large tube strips required by the KingFisher™ mL Magnetic Particle Processor.
- 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 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 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 2 Recommended scripts

Instrument	Script name
KingFisher™ Flex Magnetic Particle Processor	MagMAX_CORE_Flex.bdz
KingFisher™ 96 Magnetic Particle Processor MagMAX™ Express-96 Magnetic Particle Processor	MagMAX_CORE_KF-96.bdz
KingFisher™ Duo Prime Magnetic Particle Processor	MagMAX_CORE_DUO.bdz
KingFisher™ mL Magnetic Particle Processor	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 3 Alternate scripts without heated elution step

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

See your instrument user guide or a Thermo Fisher Scientific representative for instructions for installing the script.

3 Simple Workflow

The Simple workflow is recommended for the following sample types. The nucleic acid this purification workflow is optimized for varies by sample type; see "Recommended workflows" on page 7 for details.

- Biomed Diagnostics InPouch™ TF (*Tritrichomonas foetus*) culture
- Ear notch (triangular shape, approximately 1-cm width)
- Ear punch (circular shape, 2- to 3-mm diameter; PBS incubation)
- Milk
- Plasma
- Semen
- Serum
- Swabs—animal
- Tissue or organ
- Whole blood

Follow this procedure if you are using these instruments:

- KingFisher™ Flex Magnetic Particle Processor 96DW
- 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

Workflow: Simple

Set up the processing plates

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Prepare Bead/PK Mix

▼

Prepare Lysis/Binding Solution

▼

Prepare the sample

▼

Combine the sample with Bead/PK Mix and Lysis/Binding Solution

▼

Process samples on the instrument

Set up the processing plates

1. Set up the processing plates.

Table 4 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 Bead/PK Mix

We recommend that you prepare new Bead/PK Mix for each processing run. If necessary, you can store Bead/PK Mix at 4°C for up to one week.

1. Vortex the MagMAX™ CORE Magnetic Beads thoroughly to ensure that the beads are fully resuspended.

2. Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Magnetic Beads	20 µL
MagMAX™ CORE Proteinase K	10 µL
Total Bead/PK Mix	**30 µL**

Prepare Lysis/Binding Solution

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
Total Lysis/Binding Solution (–IPC)	**700 µL**
(*Optional*) Internal positive control (IPC), one of the following:	
VetMAX™ Xeno™ Internal Positive Control DNA	2 µL
VetMAX™ Xeno™ Internal Positive Control RNA	2 µL
Internal positive control (IPC) supplied with your VetMAX™ PCR Kit	As indicated in the instructions for the kit
Total Lysis/Binding Solution (+IPC)	**700 µL + volume of IPC**

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

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

Prepare the sample

Prepare samples according to sample type.

For...	Do this...
Biomed Diagnostics InPouch™ TF culture	Proceed with 300 µL of previously enriched culture media.
Ear notch (triangular shape, approximately 1-cm width)	1. Add one ear notch to a 5-mL specimen tube. 2. Add 2 mL of PBS, pH 7.4 to each sample. 3. Incubate at room temperature with or without shaking: • Without shaking—15 minutes • With moderate shaking—10 minutes 4. Proceed with 200 µL of supernatant.
Ear punch (circular shape, 2- to 3-mm diameter)	1. Add one ear punch to a 2-mL tube. 2. Add 200 µL of PBS, pH 7.4 to each sample. 3. Incubate at room temperature with or without shaking: • Without shaking—15 minutes • With moderate shaking—10 minutes 4. Proceed with 50–200 µL of supernatant.
Milk, plasma, serum, or whole blood	Proceed with 200 µL of sample.
Semen	1. Add 500 µL of semen to a fresh tube. 2. Centrifuge at 15,000 × *g* for 2 minutes. 3. Proceed with 200 µL of supernatant.
Swabs—animal	Follow the manufacturer's recommended protocol, or follow this procedure: 1. Break off the tip of the swab and add to a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4 to each sample. 3. Vortex for 3 minutes. 4. Proceed with 200 µL of supernatant.
Tissue or organ	1. Add the following components to a 2-mL tube: • Tissue: 20–30 mg • PBS, pH 7.4: 1 mL • PYREX™ Solid Glass Beads for Distillation Columns (3 mm): 2 beads 2. Disrupt (bead-beat) the samples in a Fisher Scientific™ Bead Mill 24 Homogenizer at 6 m/s for 45 seconds. 3. Centrifuge at 1,000 × *g* for 1 minute. 4. Proceed with 100 µL of supernatant.

Combine the sample with Bead/PK Mix and Lysis/Binding Solution

1. Invert the tube of Bead/PK Mix several times to resuspend the beads, then add 30 µL of the Bead/PK Mix to the required wells in the plate or tube strip.
2. Transfer the appropriate volume of each prepared sample to a well with Bead/PK Mix.

For...	Use...
Biomed Diagnostics InPouch™ TF culture	300 µL of supernatant
Ear notch (triangular shape, approximately 1-cm width) Semen Swabs—animal	200 µL of supernatant
Ear punch (circular shape, 2- to 3-mm diameter)	50–200 µL of supernatant
Milk, plasma, serum, or whole blood	200 µL of sample
Tissue or organ	100 µL of supernatant

3. Mix the sample with Bead/PK Mix for 2 minutes at room temperature according to your mixing method.
   - **Using a plate shaker**: shake vigorously for 2 minutes (see "(Optional) Determine the maximum plate shaker setting" on page 8).
   - **By pipetting:** pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL Magnetic Particle Processor, you must mix by pipetting.)
4. Add 700 µL of Lysis/Binding Solution to each sample-containing well or tube.
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 9).
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.

4 Complex Workflow

The Complex workflow is recommended for the following sample types. The nucleic acid this purification workflow is optimized for varies by sample type; see "Recommended workflows" on page 7 for details.

- Environmental samples
- Feces
- Oral fluid
- Swabs—environmental or fecal

Follow this procedure if you are using these instruments:

- KingFisher™ Flex Magnetic Particle Processor 96DW
- 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

Workflow: Complex

Set up the processing plates

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Prepare Bead/PK Mix

▼

Prepare the Lysis Solution

▼

Prepare the clarified lysate (tube or plate processing)

▼

Combine the clarified lysate with Bead/PK Mix and MagMAX™ CORE Binding Solution

▼

Process samples on the instrument

Set up the processing plates

1. Set up the processing plates.

Table 5 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 Bead/PK Mix

We recommend that you prepare new Bead/PK Mix for each processing run. If necessary, you can store Bead/PK Mix at 4°C for up to one week.

1. Vortex the MagMAX™ CORE Magnetic Beads thoroughly to ensure that the beads are fully resuspended.

2. Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Magnetic Beads	20 µL
MagMAX™ CORE Proteinase K	10 µL
Total Bead/PK Mix	**30 µL**

Prepare the Lysis Solution

1. Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Lysis Solution	450 µL
(*Optional*) Internal positive control (IPC), one of the following:	
VetMAX™ Xeno™ Internal Positive Control DNA	2 µL
VetMAX™ Xeno™ Internal Positive Control RNA	2 µL
Internal positive control (IPC) supplied with your VetMAX™ PCR Kit	As indicated in the instructions for the kit
Total Lysis Solution (+IPC)	**450 µL + volume of IPC**

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

(*Optional*) Store Lysis Solution at room temperature for up to 24 hours.

Prepare the clarified lysate

1. Prepare samples according to sample type.

For...	Do this...
Environmental samples Feces	1. Transfer 0.2-0.3 g of sample to a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4, then vortex vigorously for 3 minutes. 3. Centrifuge as indicated. • **For viral nucleic acid purification**: centrifuge at 15,000 × *g* for 1 minute. • **For bacterial DNA purification or concurrent purification of bacterial and viral nucleic acids**: centrifuge at 100 × *g* for 1 minute. 4. Proceed with 200 µL of supernatant.
Oral fluid	1. Briefly mix the oral fluid sample. 2. Proceed with 300 µL of sample.
Swabs—environmental or fecal	1. **Fecal samples:** swirl a clinical swab in a fecal sample. **Environmental swabs:** proceed with an environmental swab. 2. Add 1 mL of PBS, pH 7.4 to a 2-mL tube. 3. Swirl the swab in 1 mL of PBS, pH 7.4 for 5–10 seconds, removing as much sample material as possible, then discard the swab. Alternatively, break off the swab tip and leave the swab in the PBS, pH 7.4. 4. Vortex vigorously for 3 minutes, or until the sample is suspended. 5. Centrifuge as indicated. • **For viral nucleic acid purification**: centrifuge at 15,000 × *g* for 1 minute. • **For bacterial DNA purification or concurrent purification of bacterial and viral nucleic acids**: centrifuge at 100 × *g* for 1 minute. 6. Proceed with 200 µL of supernatant.

2. Add Lysis Solution, then clarify the lysate.

For...	Do this...
Processing in tubes	1. For each sample, add 450 µL of Lysis Solution to a new 2-mL tube. 2. Add the indicated volume of sample from step 1 on page 18 to the Lysis Solution. 3. Vortex vigorously for 3 minutes. 4. Centrifuge at 15,000 × *g* for 2 minutes. 5. Remove the supernatant (clarified lysate) without disturbing the pellet.
Processing in plates	1. For each sample, add 450 µL of Lysis Solution to the appropriate wells of a deep-well plate. 2. Add the indicated volume of sample from step 1 on page 18 to the Lysis Solution. 3. Seal the plate with sealing foil. 4. Shake the plate at moderate speed for 5 minutes. 5. Centrifuge at 3,000 × *g* for 5 minutes 6. Remove the supernatant (clarified lysate) without disturbing the pellet.

Combine the clarified lysate with Bead/PK Mix and MagMAX™ CORE Binding Solution

1. Invert the tube of Bead/PK Mix several times to resuspend the beads, then add 30 µL of the Bead/PK Mix to the required wells in the plate or tube strip.

2. Transfer the appropriate volume of each clarified lysate (see "Prepare the clarified lysate" on page 18) to a well with the Bead/PK Mix.

For...	Use...
Oral fluid	600 µL
Environmental samples, fecal samples, and swabs	500 µL

3. Mix the sample with Bead/PK Mix for 2 minutes at room temperature according to your mixing method.

   - **Using a plate shaker**: shake vigorously for 2 minutes (see "(Optional) Determine the maximum plate shaker setting" on page 8).
   - **By pipetting:** pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL Magnetic Particle Processor, you must mix by pipetting.)

4. Add 350 µL of MagMAX™ CORE Binding Solution.

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 9).
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.

5 Digestion Workflow

The Digestion workflow is recommended for the following sample types. The nucleic acid this purification workflow is optimized for varies by sample type; see "Recommended workflows" on page 7 for details. The Digestion workflow is not recommended for purification of RNA.

- Environmental samples
- Feces
- Hair follicles
- Swabs—environmental or fecal
- Tissue or organ

Follow this procedure if you are using these instruments:

- KingFisher™ Flex Magnetic Particle Processor 96DW
- 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

Workflow: Digestion

Set up the processing plates

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Prepare the Lysis/Binding Solution

▼

Prepare PK Solution

▼

Treat the samples with PK Solution (tube or plate processing)

▼

Combine Proteinase K-treated samples with beads and Lysis/Binding Solution

▼

Process samples on the instrument

Set up the processing plates

1. Set up the processing plates.

Table 6 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 the Lysis/Binding Solution

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
Total Lysis/Binding Solution (–IPC)	**700 µL**
(*Optional*) Internal positive control (IPC), one of the following:	
VetMAX™ Xeno™ Internal Positive Control DNA	2 µL
VetMAX™ Xeno™ Internal Positive Control RNA	2 µL
Internal positive control (IPC) supplied with your VetMAX™ PCR Kit	As indicated in the instructions for the kit
Total Lysis/Binding Solution (+IPC)	**700 µL + volume of IPC**

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

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

Prepare PK Solution

Prepare PK Solution immediately before use.

1. Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
PK Buffer for MagMAX™-96 DNA Multi-Sample Kit	90 µL
MagMAX™ CORE Proteinase K	10 µL
Total PK Solution	**100 µL**

2. Invert the tube several times to mix, then centrifuge briefly to collect contents at the bottom of the tube.

3. Proceed immediately to the next step:

   - **For tube processing**—proceed to "Treat the samples with PK Solution (tube processing)" on page 24.
   - **For plate processing**—proceed to "Treat the samples with PK Solution (plate processing)" on page 26.

Treat the samples with PK Solution

Treat the samples with PK Solution (tube processing)

Treat samples with PK Solution according to the sample type.

Sample type	Procedure
Environmental samples Feces	1. Transfer 0.2–0.3 g of sample to a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4, then vortex vigorously for 3 minutes. 3. Centrifuge at 100 × *g* for 1 minute. 4. Transfer 200 µL of the supernatant to a new tube. 5. Add 100 µL of PK Solution to the transferred supernatant, then vortex briefly to mix. 6. Incubate for 30 minutes at 55°C. 7. Centrifuge at 15,000 × *g* for 2 minutes. 8. Proceed with 200 µL of digested sample.
Hair follicles	1. Place 10–15 hair follicles in a 2-mL tube. 2. Add 100 µL of PK Solution to the sample. 3. Incubate for 30 minutes at 55°C. 4. Centrifuge briefly to collect the contents to the bottom of the tube. 5. Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 µL.
Swabs—environmental or fecal	1. **Fecal samples**: swirl a clinical swab in a fecal sample. **Environmental swabs**: proceed with an environmental swab. 2. Add 1 mL of PBS, pH 7.4 to a 2-mL tube. 3. Swirl the swab in the PBS, pH 7.4 for 5–10 seconds, removing as much sample material as possible, then discard the swab. Alternatively, break off the swab tip and leave the swab in the PBS, pH 7.4. 4. Vortex vigorously for 3 minutes, or until the sample is suspended. 5. Centrifuge at 100 × *g* for 1 minute. 6. Transfer 200 µL of the supernatant to a new tube. 7. Add 100 µL of PK Solution to the transferred supernatant, then vortex briefly to mix. 8. Incubate for 30 minutes at 55°C. 9. Centrifuge at 15,000 × *g* for 2 minutes. 10. Proceed with 200 µL of digested sample.
Tissue or organ	1. Transfer 20–30 mg of tissue to a 2-mL tube. 2. Add 100 µL of PK Solution to the sample. 3. Incubate for 2 hours at 55°C. 4. Centrifuge briefly to collect the contents to the bottom of the tube. 5. Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 µL.

Sample type	Procedure
	Use a P1000 pipette tip to transfer the viscous sample.

Treat the samples with PK Solution (plate processing)

Treat samples with PK Solution according to the sample type.

Sample type	Procedure
Environmental samples Feces	1. Transfer 0.2–0.3 g of sample to a well of a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4 to each sample, then vortex vigorously for 3 minutes. 3. Centrifuge at 100 × *g* for 1 minute. 4. Transfer 200 µL of each supernatant to a deep-well plate. 5. Add 100 µL of PK Solution to each transferred supernatant, then pipet up and down to mix. 6. Seal the plate with sealing foil. 7. Incubate for 30 minutes at 55°C. 8. Centrifuge at 3,000 × *g* for 5 minutes. 9. Proceed with 200 µL of digested sample.
Hair follicles	1. Place 10–15 hair follicles in a well of a deep-well plate. 2. Add 100 µL of PK Solution to each sample. 3. Seal the plate with sealing foil. 4. Incubate for 30 minutes at 55°C. 5. Centrifuge briefly to collect the contents to the bottom of the plate. 6. Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 µL.
Swabs—environmental or fecal	1. **Fecal samples**: swirl a clinical swab in a fecal sample. **Environmental swabs**: proceed with an environmental swab. 2. Add 1 mL of PBS, pH 7.4 to a 2-mL tube. 3. Swirl the swab in the PBS, pH 7.4 for 5–10 seconds, removing as much sample material as possible, then discard the swab. Alternatively, break off the swab tip and leave the swab in the PBS, pH 7.4. 4. Vortex vigorously for 3 minutes, or until the samples are suspended. 5. Centrifuge at 100 × *g* for 1 minute. 6. Transfer 200 µL of each supernatant to a deep-well plate. 7. Add 100 µL of PK Solution to each transferred supernatant, then pipet up and down to mix. 8. Seal the plate with sealing foil. 9. Incubate for 30 minutes at 55°C. 10. Centrifuge at 3,000 × *g* for 2 minutes. 11. Proceed with 200 µL of digested sample.
Tissue or organ samples	1. Transfer 20–30 mg of tissue to a well of a deep-well plate. 2. Add 100 µL of PK Solution to each sample. 3. Seal the plate with sealing foil. 4. Incubate for 2 hours at 55°C.

Sample type	Procedure
	5. Centrifuge briefly to collect the contents to the bottom of the plate. 6. Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 µL. Use a P1000 pipette tip to transfer the viscous sample.

Combine Proteinase K-treated samples with beads and Lysis/Binding Solution

1. Vortex the tube of MagMAX™ CORE Magnetic Beads several times to resuspend the beads, then add 20 µL of the beads to the required wells in the plate or tube strip.

   Note: Do not use Bead/PK Mix.

2. Add the appropriate volume of each Proteinase K-treated sample to a well with beads.

For...	Use...
Environmental samples, feces Swabs	200 µL
Hair follicles Tissue or organ samples	Up to 100 µL

3. Mix the sample with beads for 2 minutes at room temperature according to your mixing method.

   - **Using a plate shaker**: shake vigorously for 2 minutes (see "(Optional) Determine the maximum plate shaker setting" on page 8).
   - **By pipetting:** pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL Magnetic Particle Processor, you must mix by pipetting.)

4. Add 700 µL of Lysis/Binding Solution 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 9).
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.

6 Lysis Incubation Workflow

The Lysis Incubation workflow is recommended for ear punches that require processing:

- With an extended lysis step before nucleic acid isolation.
- By addition of punches directly to a lysis solution.

Follow this procedure if you are using these instruments:

- KingFisher™ Flex Magnetic Particle Processor 96DW
- 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

Workflow: Lysis Incubation

The Lysis Incubation workflow can be performed with a 15-min or an overnight incubation in Lysis Solution. If samples are incubated overnight, set up the processing plates and prepare Lysis/Binding/Bead Mix after the incubation is complete.

15-min incubation

Set up the processing plates

▼

Prepare Lysis/Binding/Bead Mix

▼

Prepare the ear punch lysate

(15 min incubation)

▼

Combine the lysate with Proteinase K and Lysis/ Binding/Bead Mix

▼

Process samples on the instrument

Overnight incubation

Prepare the ear punch lysate

(overnight incubation)

▼

Set up the processing plates

▼

Prepare Lysis/Binding/Bead Mix

▼

Combine the lysate with Proteinase K and Lysis/ Binding/Bead Mix

▼

Process samples on the instrument

Set up the processing plates

1. Set up the processing plates.

Table 7 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/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:	
VetMAX™ Xeno™ Internal Positive Control DNA	2 µL
VetMAX™ Xeno™ Internal Positive Control RNA	2 µL
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**

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

Prepare the ear punch lysate

1. Add 300 µL of MagMAX™ CORE Lysis Solution to each ear punch.
2. Incubate without shaking at room temperature for the desired time.
   - For 15 minutes
   - For 16–18 hours (overnight)

3. Proceed with individual or pooled supernatants.

For...	Do this...
Individual samples	Proceed with 250 µL of supernatant.
Pooled samples	1. Combine 50 µL of individual supernatants in a 2-mL microcentrifuge tube. 2. If the volume of pooled supernatants is less than 250 µL, add MagMAX™ CORE Lysis Solution to a total of 250 µL. 3. Vortex briefly to mix the pooled samples. 4. Proceed with 250 µL of pooled supernatant. For example: • For a pool of 10 samples, the combined volume is 500 µL (10 × 50 µL). Proceed to the next step with 250 µL of the pool. • For a pool of 4 samples, the combined volume is 200 µL (4 × 50 µL). Add 50 µL of MagMAX™ CORE Lysis Solution and proceed to the next step with the 250-µL pool.
Individual analysis of a positive pool	Proceed with the remaining supernatant of each individual sample in the positive pool. The volume may be less than 250 µL.

Store individual and pooled lysates for retesting: up to 48 hours at room temperature, or longer term below –16°C.

Combine the lysate with Proteinase K and Lysis/Binding/Bead Mix

1. Add 10 µL of MagMAX™ CORE Proteinase K to the required wells in the plate or tube strip.
2. Add 250 µL of individual or pooled supernatant.
3. Mix the supernatant with Proteinase K by pipetting up and down several times, then incubate for 2 minutes at room temperature.
4. Invert the tube of Lysis/Binding/Bead Mix several times to resuspend the beads, then add 720 µL of Lysis/Binding/Bead 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 9).
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
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.
Poor or no RNA or DNA signal (that is, the C_t value is higher than expected) In test samples, the C_t value of the IPC target is outside of the validated value range.	There are inhibitors 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 RT-PCR or 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 IPC result, the obtained result is validated. • If the diluted nucleic acid is negative for the target with a non-compliant IPC result, the obtained result is not validated. In this case, dilute the original biological sample 1:10 in 1X PBS, and repeat the purification and PCR. If the result is still not validated, repeat the purification and PCR on a new biological sample.
		Repeat the purification using the Complex workflow.
	Samples with high amounts of nucleic acid, such as tissue, avian blood, and bacterial cultures, can saturate the magnetic beads. Bead saturation reduces nucleic acid recovery.	For the samples that show reduced recovery of the internal positive control RNA or DNA, dilute samples 1:2 1:4, 1:8, and 1:16 in PBS. Use the dilution that shows the best internal positive control recovery.
	The IPC DNA or RNA did not bind efficiently to the magnetic beads, due to extracellular material in the sample.	Add MagMAX™ CORE Magnetic Beads to the Lysis/Binding Solution, instead of preparing Bead/PK Mix or adding beads directly to the sample.
Poor yield of viral RNA from tissue, fecal or environmental samples, or swabs	The Digestion workflow was used for viral nucleic acid purification.	Follow the appropriate workflow. See "Recommended workflows" on page 7.

Observation	Possible cause	Recommended action
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.
Positive samples are clustered in the PCR plate	High-titer samples (exhibiting a low or early C_t) have contaminated nearby wells. If the same plate layout is used from nucleic acid purification through PCR, it can be difficult to tell whether contamination occurred during nucleic acid purification or during PCR.	Repeat the nucleic acid purification of the positive or suspect samples without the high-titer samples. Be careful to avoid splashing when pipetting the reagents or samples.

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 8 Materials required for processing on the KingFisher™ Duo Prime Magnetic Particle Processor

Item	Source
KingFisher™ Duo Prime Magnetic Particle Processor	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 9 Materials required for processing on the KingFisher™ mL Magnetic Particle Processor

Item	Source
KingFisher™ mL Magnetic Particle Processor	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 Magnetic Particle Processor, 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 10 Plate setup: KingFisher™ Duo Prime Magnetic Particle Processor

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 11 Tube strip setup: KingFisher™ mL Magnetic Particle Processor

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 14.

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
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

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- Product documentation, including:
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  - 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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