

# MagMAX™ CORE Nucleic Acid Purification Kit

## USER GUIDE

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

for use with:

KingFisher™ Flex Purification System

KingFisher™ Duo Prime Purification System

KingFisher™ mL Purification System

**Catalog Numbers** A32700, A32702

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**Revision** E



Revision history: MAN0015944 E (English)

Revision	Date	Description
E	21 March 2025	<ul style="list-style-type: none"><li>• A sample type was corrected (see "Prepare the sample" on page 23).</li><li>• Minor reorganization of the required materials section for style and clarity, and minor edits for style and consistency.</li></ul>
D00	25 April 2024	<ul style="list-style-type: none"><li>• New alternate workflows for whole blood and semen samples were added (see Chapter 4, "Whole Blood Workflow" and Chapter 5, "Semen Workflow").</li><li>• The MagMAX™ Express-96 was removed due to product discontinuation.</li><li>• Minor edits were made for style and consistency.</li></ul>
C.0	13 December 2017	<ul style="list-style-type: none"><li>• Updated the usage statement on the front cover.</li><li>• Minor reorganization of the required materials section for style and clarity.</li><li>• Minor corrections to product names. Minor edits for style and consistency.</li></ul>
B.0	30 June 2017	<ul style="list-style-type: none"><li>• Combined and renamed workflows:<ul style="list-style-type: none"><li>– Simple: formerly Workflows A and C</li><li>– Complex: formerly Workflow B</li><li>– Digestion: formerly Workflow D</li></ul></li><li>• Added new workflow: Lysis Incubation.</li><li>• Added plate processing of samples in the Digestion Workflow.</li><li>• Added list of instrument scripts. Reorganized into chapters for better navigation and clarity.</li></ul>
A.0	22 December 2016	New document created for MagMAX™ CORE Nucleic Acid Purification Kit.

The information in this guide is subject to change without notice.

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# Product information

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

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## 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
- 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 9.

## Contents and storage

Table 1 MagMAX™ CORE Nucleic Acid Purification Kit

Contents	Cat. No. <a href="#">A32700</a> (100 reactions)	Cat. No. <a href="#">A32702</a> (500 reactions)	Storage
MagMAX™ CORE Lysis Solution <sup>[1]</sup>	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	

<sup>[1]</sup> Available for purchase separately (Cat. No. A32837).

## Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

**Table 2** Materials required for all workflows performed on the KingFisher™ Flex<sup>[1]</sup>

Item	Source
<b>Instrument and equipment</b>	
KingFisher™ Flex Purification System See page 45 for other compatible instruments.	Contact your local sales office.
Benchtop microcentrifuge capable of 15,000 × g	MLS
Laboratory mixer, Vortex or equivalent	MLS
<b>Reagents</b>	
PBS (1X), pH 7.4 <sup>[2]</sup>	<a href="#">10010023</a>
<b>(Optional) Internal positive control (IPC), one of the following:</b>	
VetMAX™ Xeno™ Internal Positive Control DNA	<a href="#">A29764</a>
VetMAX™ Xeno™ Internal Positive Control RNA	<a href="#">A29763</a>
IPC supplied with your VetMAX™ PCR Kit	<a href="https://www.thermofisher.com">thermofisher.com</a>
<b>Tubes, plates, and other consumables<sup>[3]</sup></b>	
5-mL tubes, or equivalent	MLS
2-mL tubes, or equivalent	MLS
Adhesive PCR Plate Foils, or equivalent	<a href="#">AB0626</a>
KingFisher™ Flex Microtiter Deep-Well 96 plates, 50 plates	<a href="#">95040460</a>
KingFisher™ 96 KF microplates (200 µL), 48 plates	<a href="#">97002540</a>
KingFisher™ 96 tip comb for deep-well magnets, 100 combs	<a href="#">97002534</a>

<sup>[1]</sup> See Table 4 and Table 5 for additional materials required for the Simple, Digestion, Whole Blood and Semen workflows.

<sup>[2]</sup> Not required for the Lysis Incubation workflow.

<sup>[3]</sup> See page 45 for other the consumables of other compatible instruments.

**Table 3** Optional equipment

Item	Source
Biotang Inc Microplate Shaker, or equivalent titer plate shaker (for mixing beads with samples; all workflows)	Fisher Scientific™ 50-751-4965
Benchtop centrifuge with plate adaptors (for lysate preparation in plates; Complex and Digestion workflows)	MLS

**Table 4 Additional materials required for the Simple workflow (tissue samples only)**

Item	Source
Fisherbrand™ Bead Mill 24 Homogenizer	Fisher Scientific™ <a href="#">15-340-163</a>
PYREX™ Solid Glass Beads for Distillation Columns (3 mm)	Fisher Scientific™ <a href="#">11-312-10A</a>

**Table 5 Additional materials required for the Digestion, alternate Whole Blood, and Semen Workflows**

Item	Source
Laboratory benchtop incubator, or equivalent	MLS
PK Buffer for MagMAX™-96 DNA Multi-Sample Kit	<a href="#">4489111</a>
Phosphate-buffered saline (PBS, 1X), sterile-filtered	J61196.AP

## Recommended workflows

**Note:** For tough-to-lyse bacteria, for example, *M. paratuberculosis* (MAP), use the MagMAX™ CORE Mechanical Lysis Module (Cat. Nos. A32836, [A37487](#)).

Sample matrix	Nucleic acid	Recommended workflow
<ul style="list-style-type: none"> <li>Ear punch (circular shape, 2- to 3-mm diameter) in Lysis Solution</li> </ul>	Viral nucleic acid	Lysis Incubation <sup>[1]</sup> (page 39)
<ul style="list-style-type: none"> <li>Ear punch (circular shape, 2- to 3-mm diameter) in PBS</li> <li>Ear notch (triangular shape, approximately 1-cm width)</li> <li>Milk</li> <li>Plasma</li> <li>Serum</li> </ul>	<ul style="list-style-type: none"> <li>Viral nucleic acid</li> <li>Bacterial DNA</li> </ul>	Simple (page 12)
<ul style="list-style-type: none"> <li>Biomed Diagnostics InPouch™ TF (<i>Tritrichomonas foetus</i>) culture</li> </ul>	<i>Tritrichomonas foetus</i> DNA	
<ul style="list-style-type: none"> <li>Semen<sup>[2]</sup></li> </ul>	Viral nucleic acid	
<ul style="list-style-type: none"> <li>Swabs—animal</li> <li>Whole blood<sup>[3]</sup></li> </ul>	<ul style="list-style-type: none"> <li>Viral nucleic acid</li> <li>Genomic DNA</li> </ul>	
<ul style="list-style-type: none"> <li>Tissue or organ</li> </ul>	<ul style="list-style-type: none"> <li>Viral nucleic acid</li> <li>Bacterial DNA<sup>[4]</sup></li> </ul>	
<ul style="list-style-type: none"> <li>Hair follicles</li> </ul>	Genomic DNA	Digestion (page 31)
<ul style="list-style-type: none"> <li>Environmental samples</li> <li>Feces</li> <li>Swabs—environmental or fecal</li> </ul>	<ul style="list-style-type: none"> <li>Bacterial DNA</li> <li>Viral nucleic acid</li> <li>Bacterial DNA<sup>[4]</sup></li> </ul>	
<ul style="list-style-type: none"> <li>Oral fluid</li> </ul>	<ul style="list-style-type: none"> <li>Viral nucleic acid</li> <li>Bacterial DNA</li> </ul>	Complex (page 25)
<ul style="list-style-type: none"> <li>Semen</li> </ul>	Viral nucleic acid	Semen (page 21)
<ul style="list-style-type: none"> <li>Whole blood</li> </ul>	<ul style="list-style-type: none"> <li>Viral nucleic acid</li> <li>Genomic DNA</li> </ul>	Whole Blood (page 18)

<sup>[1]</sup> Recommended if overnight incubation is required.

<sup>[2]</sup> If you have encountered low nucleic acid recovery, follow the Semen Workflow (page 21).

<sup>[3]</sup> If you have encountered bead coagulation or aggregation with whole blood samples, follow the Whole Blood Workflow (page 18).

<sup>[4]</sup> If concurrent isolation of viral nucleic acid and bacterial DNA is not required, use the Digestion workflow.



# Before you begin

## Procedural guidelines

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

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**Note:** Do not use a plate shaker with the tube strips that are required by the KingFisher™ mL instrument.

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- To help 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 help 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 nuclease-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, use the following steps to 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](https://www.thermofisher.com), search by catalogue number), scroll to the **Product Literature** section.
2. Locate and download the latest version of the appropriate file, then download the latest version of the `MagMAX_CORE` script for your instrument.

**Table 6 Recommended scripts**

Instrument	Script name
KingFisher™ Flex	MagMAX_CORE_Flex.bdz
	MagMAX_CORE_Flex_Express.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 liquid during the elution step.

**Table 7 Alternate scripts without heated elution step**

Instrument	Script name
KingFisher™ Flex	MagMAX_CORE_Flex_no_heat.bdz
KingFisher™ Duo Prime	MagMAX_CORE_DUO_no_heat.bdz
KingFisher™ mL	MagMAX_CORE_mL_no_heat.bdz

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

# 3

## Simple Workflow

The Simple Workflow is recommended and optimized for the following sample types.

- 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

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### IMPORTANT!

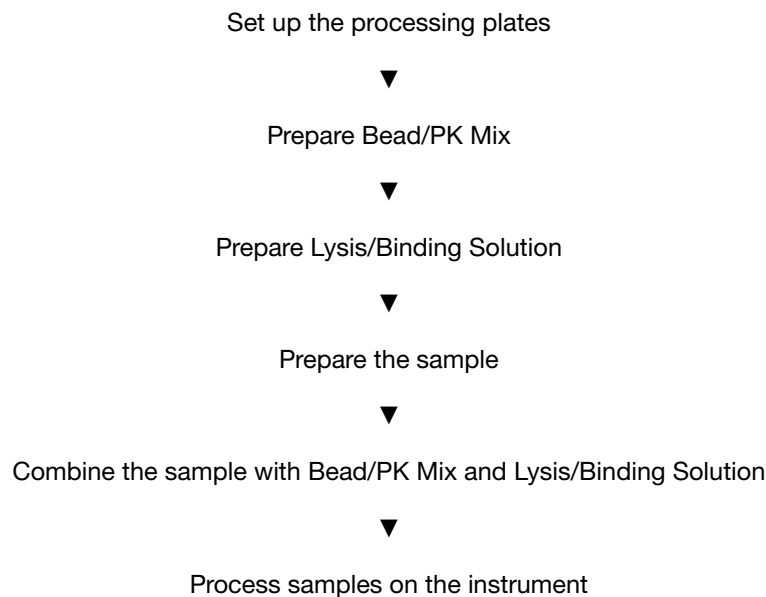
- If you have encountered bead coagulation or aggregation with whole blood samples, follow the Whole Blood Workflow (page 18).
  - If you have encountered low nucleic acid recovery, follow the Semen Workflow (page 21).
- 

Follow this procedure if you are using the KingFisher™ Flex instrument.

If you are using the following instruments, follow the procedure in Appendix B, “Purification with the KingFisher™ Duo Prime or KingFisher™ mL instrument”:

- KingFisher™ Duo Prime
- KingFisher™ mL

## Workflow: Simple



## Set up the processing plates

1. Set up the processing plates.

Table 8 Plate setup: KingFisher™ Flex instrument

Plate ID	Plate position <sup>[1]</sup>	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.	

<sup>[1]</sup> 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 1 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 (recommended).

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

## Prepare Lysis/Binding Solution

- 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
<b>Total Lysis/Binding Solution (-IPC)</b>	<b>700 µL</b>
<i>(Optional)</i> 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
<b>Total Lysis/Binding Solution (+IPC)</b>	<b>700 µL + volume of IPC</b>

- 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.

Option	Action
Biomed Diagnostics InPouch™ TF culture	Proceed with 300 µL of previously enriched culture media.
Ear notch (triangular shape, approximately 1-cm width)	<ol style="list-style-type: none"> <li>Add one ear notch to a 5-mL specimen tube.</li> <li>Add 2 mL of PBS (1X), pH 7.4 to each sample.</li> <li>Incubate at room temperature with or without shaking: <ul style="list-style-type: none"> <li>Without shaking—15 minutes</li> <li>With moderate shaking—10 minutes</li> </ul> </li> <li>Proceed with 200 µL of supernatant.</li> </ol>
Ear punch (circular shape, 2- to 3-mm diameter)	<ol style="list-style-type: none"> <li>Add one ear punch to a 2-mL tube.</li> <li>Add 200 µL of PBS (1X), pH 7.4 to each sample.</li> <li>Incubate at room temperature with or without shaking: <ul style="list-style-type: none"> <li>Without shaking—15 minutes</li> <li>With moderate shaking—10 minutes</li> </ul> </li> <li>Proceed with 50–200 µL of supernatant.</li> </ol>
Milk, plasma, serum, or whole blood <sup>[1]</sup>	Proceed with 200 µL of sample.

(continued)

Option	Action
Semen <sup>[2]</sup>	<ol style="list-style-type: none"> <li>1. Add 500 µL of semen to a fresh tube.</li> <li>2. Centrifuge at 15,000 × <i>g</i> for 2 minutes.</li> <li>3. Proceed with 200 µL of supernatant.</li> </ol>
Swabs—animal	<p>Follow the manufacturer's recommended protocol, or follow this procedure:</p> <ol style="list-style-type: none"> <li>1. Break off the tip of the swab and add to a 2-mL tube.</li> <li>2. Add 1 mL of PBS (1X), pH 7.4 to each sample.</li> <li>3. Vortex for 3 minutes.</li> <li>4. Proceed with 200 µL of supernatant.</li> </ol>
Tissue or organ	<ol style="list-style-type: none"> <li>1. Add the following components to a 2-mL tube: <ul style="list-style-type: none"> <li>• Tissue—20 to 30 mg</li> <li>• PBS (1X), pH 7.4—1 mL</li> <li>• PYREX™ Solid Glass Beads for Distillation Columns (3 mm)—2 beads</li> </ul> </li> <li>2. Disrupt (bead-beat) the samples in a Fisherbrand™ Bead Mill 24 Homogenizer at 6 m/s for 45 seconds.</li> <li>3. Centrifuge at 1,000 × <i>g</i> for 1 minute.</li> <li>4. Proceed with 100 µL of supernatant.</li> </ol>

<sup>[1]</sup> If using the alternate Whole blood Workflow, see “Prepare the sample” on page 20.

<sup>[2]</sup> If using the alternate Semen Workflow, see “Prepare the sample” on page 23.

## 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.

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

3. Mix the sample with the Bead/PK Mix for 2 minutes at room temperature according to your mixing method.
  - **Using a plate shaker**—Shake vigorously for 2 minutes (see “Determine the maximum plate shaker setting” on page 10).
  - **By pipetting**—Pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL instrument, you must mix by pipetting.)
4. Add 700 µL of Lysis/Binding Solution to each sample-containing well or tube.
5. Immediately proceed to load the samples into the instrument (next section).

**Note:** To increase efficiency of extraction setup, add the beads to the Lysis/Binding Mix. The Bead Mix step can be eliminated and Proteinase K can be added directly to the sample wells. See table below for setup.

Item	Amount per sample
MagMAX™ CORE Lysis Solution	350 µL
MagMAX™ CORE Binding Solution	350 µL
MagMAX™ CORE Magnetic Beads	20 µL
<b>Total Lysis/Binding Solution (–IPC)</b>	<b>720 µL</b>
<b>(Optional) Internal positive control (IPC), one of the following:</b>	
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
<b>Total Lysis/Binding Solution (+IPC)</b>	<b>720 µL + volume of IPC</b>

## Process samples on the instrument

1. Select the appropriate script on the instrument (see “Download and install the script” on page 11).
2. Start the run, then load the prepared plates or tube strips in the appropriate positions when prompted by the instrument.

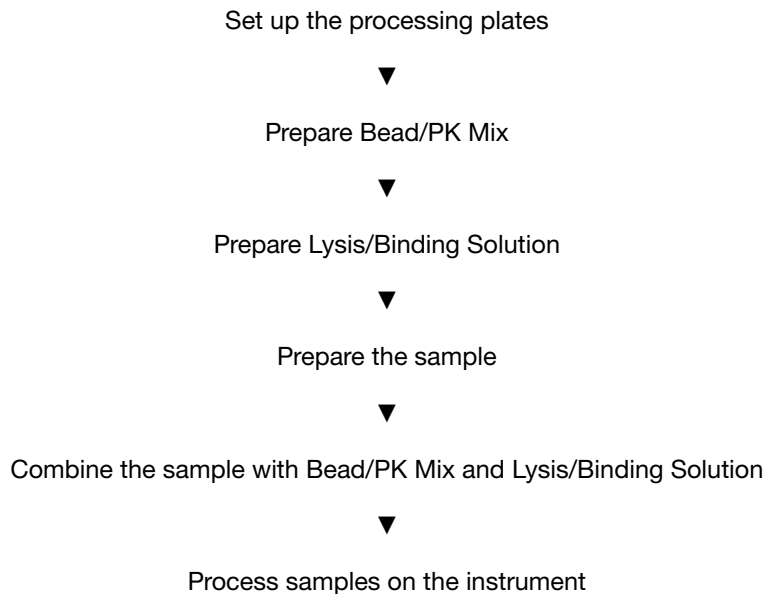
Store purified nucleic acid on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

# 4

## Whole Blood Workflow

The Whole Blood Workflow is recommended for users experiencing bead aggregation or coagulation with the Simple Workflow. This workflow is designed to minimize bead aggregation and coagulation while maintaining sensitivity for downstream applications.

### Workflow: Whole Blood



## Set up the processing plates

1. Set up the processing plates.

**Table 9 Plate setup: KingFisher™ Flex instrument**

Plate ID	Plate position <sup>[1]</sup>	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.	

<sup>[1]</sup> 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 PK/PBS Mix

Prepare new PK/PBS Mix for each processing run.

Combine the following components for the required number of samples, plus 10% overage (recommended).

Component	Volume per sample
MagMAX™ CORE Proteinase K	10 µL
1X PBS	190 µL
<b>Total PK/PBS Mix</b>	<b>200 µL</b>

## Prepare Lysis/Binding/Bead Mix

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 (recommended).

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

*(continued)*

Component	Volume per sample
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
<b>Total Lysis/Binding Mix (+IPC)</b>	<b>700 µL + volume of IPC</b>

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

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

## Prepare the sample

Prepare samples and controls as described.

Sample type	Action
Whole blood	Proceed with 100 µL of sample.
Negative Extraction Control (NEC)	Prepare at least 1 NEC

## Combine the sample with PK/PBS Mix and Lysis/Binding/Bead Mix

- Vortex the tube of PK/PBS Mix to resuspend the beads, then add 200 µL of the PK/PBS Mix to the required wells in the plate or tube strip.
- Transfer 100 µL of each prepared whole blood sample to a well with PK/PBS Mix, then vortex to mix.
- Incubate for 5 minutes at room temperature.
- Add 720 µL of Lysis/Binding/Bead Mix to each sample-containing well or tube.
- Immediately proceed to load the samples into the instrument (next section).

## Process samples on the instrument

- Select the appropriate script on the instrument (see “Download and install the script” on page 11).
- Start the run, then load the prepared plates or tube strips in the appropriate positions when prompted by the instrument.

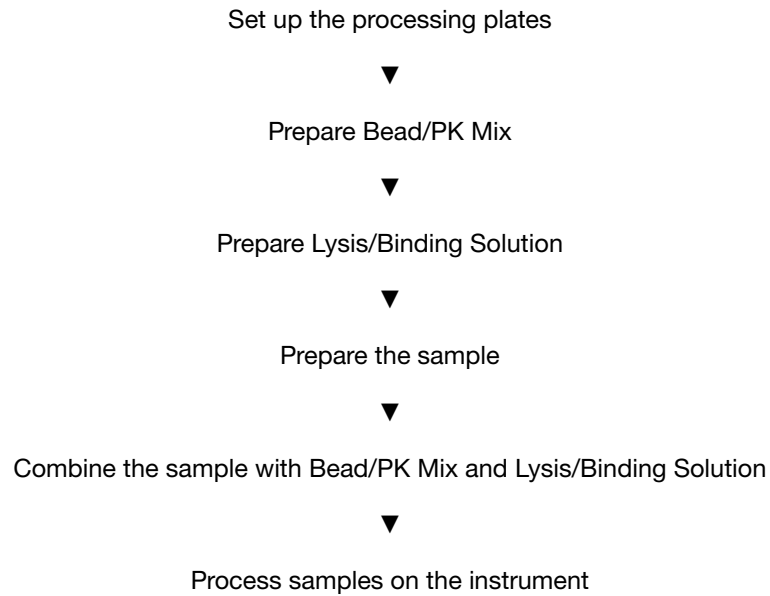
Store purified nucleic acid on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.



# Semen Workflow

The Semen Workflow is recommended for users experiencing lower nucleic acid recovery with the Simple Workflow. This workflow is designed to maximize pathogen recovery while maintaining or increasing sensitivity for downstream applications.

## Workflow: Semen



## Set up the processing plates

1. Set up the processing plates.

Table 10 Plate setup: KingFisher™ Flex instrument

Plate ID	Plate position <sup>[1]</sup>	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.	

<sup>[1]</sup> 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 PK/PBS Mix

Prepare new PK/PBS Mix for each processing run.

Combine the following components for the required number of samples, plus 10% overage (recommended).

Component	Volume per sample
MagMAX™ CORE Proteinase K	10 µL
1X PBS	200 µL
<b>Total PK/PBS Mix</b>	<b>210 µL</b>

## Prepare Binding/Bead Mix

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 (recommended).

Component	Volume per sample
MagMAX™ CORE Binding Solution	400 µL
MagMAX™ CORE Magnetic Beads	20 µL
<b>Total Binding/Bead Mix (–IPC)</b>	<b>420 µL</b>
<b>(Optional) Internal positive control (IPC), one of the following:</b>	
VetMAX™ Xeno™ Internal Positive Control DNA	2 µL
VetMAX™ Xeno™ Internal Positive Control RNA	2 µL

(continued)

Component	Volume per sample
Internal positive control (IPC) supplied with your VetMAX™ PCR Kit	As indicated in the instructions for the kit
<b>Total Binding/Bead Mix (+IPC)</b>	<b>420 µL + volume of IPC</b>

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

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

## Prepare the sample

Prepare samples and controls as described.

Sample type	Action
Semen	Proceed with 300 µL of sample.
Negative Extraction Control (NEC)	Prepare at least 1 NEC

## Combine the sample with PK/PBS Mix and Binding/Bead Mix

- Vortex the tube of MagMAX™ CORE Lysis Solution , then add 400 µL of the MagMAX™ CORE Lysis Solution to the required wells in the plate or tube strip.
- Transfer 300 µL of each semen sample to a well with MagMAX™ CORE Lysis Solution , then vortex to mix.
- Add 210 µL of PK/PBS Mix to each sample-containing well or tube, then vortex to mix.
- Incubate for 30 minutes at 70°C.
- Centrifuge the plate to collect the contents.
- Transfer 600 µL to a clean deep well plate.
- Add 420 µL of Binding/Bead Mix to each sample-containing well.
- Immediately proceed to load the samples into the instrument (next section).

## Process samples on the instrument

1. Select the appropriate script on the instrument (see “Download and install the script” on page 11).
2. Start the run, then load the prepared plates or tube strips in the appropriate positions when prompted by the instrument.

Store purified nucleic acid on ice for immediate use, at  $-20^{\circ}\text{C}$  for up to 1 month, or at  $-80^{\circ}\text{C}$  for long-term storage.



# Complex Workflow

The Complex Workflow is recommended and optimized for the following sample types.

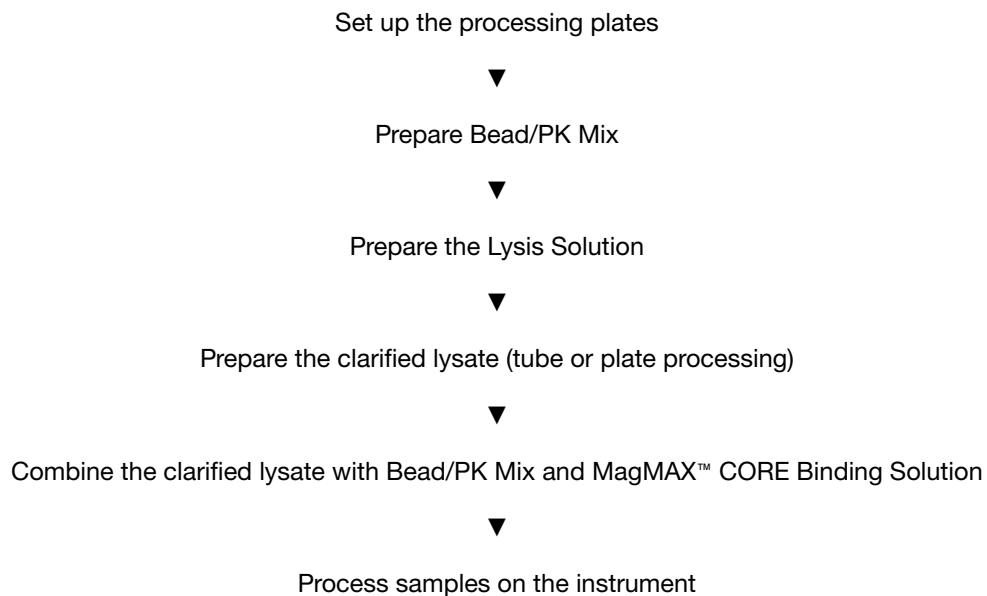
- Environmental samples
- Feces
- Oral fluid
- Swabs—environmental or fecal

Follow this procedure if you are using the KingFisher™ Flex instrument.

If you are using the following instruments, follow the procedure in Appendix B, “Purification with the KingFisher™ Duo Prime or KingFisher™ mL instrument”:

- KingFisher™ Duo Prime
- KingFisher™ mL

## Workflow: Complex



## Set up the processing plates

1. Set up the processing plates.

**Table 11 Plate setup: KingFisher™ Flex instrument**

Plate ID	Plate position <sup>[1]</sup>	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.	

<sup>[1]</sup> 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 1 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 (recommended).

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

## 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
<i>(Optional)</i> 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
<b>Total Prepared Lysis Solution (+IPC)</b>	<b>450 µL + volume of IPC</b>

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

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

## Prepare the clarified lysate

### 1. Prepare samples according to sample type.

Sample type	Action
Environmental samples Feces	<ol style="list-style-type: none"> <li>Transfer 0.2-0.3 g of sample to a 2-mL tube.</li> <li>Add 1 mL of PBS (1X), pH 7.4, then vortex vigorously for 3 minutes.</li> <li>Centrifuge as indicated. <ul style="list-style-type: none"> <li><b>For viral nucleic acid purification</b>—centrifuge at 15,000 × <i>g</i> for 1 minute.</li> <li><b>For bacterial DNA purification or concurrent purification of bacterial and viral nucleic acids</b>—centrifuge at 100 × <i>g</i> for 1 minute.</li> </ul> </li> <li>Proceed with 200 µL of supernatant.</li> </ol>
Oral fluid	<ol style="list-style-type: none"> <li>Briefly mix the oral fluid sample.</li> <li>Proceed with 300 µL of sample.</li> </ol>
Swabs— environmental or fecal	<ol style="list-style-type: none"> <li><b>Fecal samples</b>—swirl a clinical swab in a fecal sample. <b>Environmental swabs</b>—proceed with an environmental swab.</li> <li>Add 1 mL of PBS (1X), pH 7.4 to a 2-mL tube.</li> <li>Swirl the swab in 1 mL of PBS (1X), 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 (1X), pH 7.4.</li> <li>Vortex vigorously for 3 minutes, or until the sample is suspended.</li> <li>Centrifuge as indicated. <ul style="list-style-type: none"> <li><b>For viral nucleic acid purification</b>—centrifuge at 15,000 × <i>g</i> for 1 minute.</li> <li><b>For bacterial DNA purification or concurrent purification of bacterial and viral nucleic acids</b>—centrifuge at 100 × <i>g</i> for 1 minute.</li> </ul> </li> <li>Proceed with 200 µL of supernatant.</li> </ol>

2. Add Prepared Lysis Solution, then clarify the lysate.

Option	Action
Processing in tubes	<ol style="list-style-type: none"> <li>For each sample, add 450 µL of Prepared Lysis Solution to a new 2-mL tube.</li> <li>Add the indicated volume of sample from step 1 on page 28 to the Prepared Lysis Solution.</li> <li>Vortex vigorously for 3 minutes.</li> <li>Centrifuge at 15,000 × <i>g</i> for 2 minutes.</li> <li>Remove the supernatant (clarified lysate) without disturbing the pellet.</li> </ol>
Processing in plates	<ol style="list-style-type: none"> <li>For each sample, add 450 µL of Prepared Lysis Solution to the appropriate wells of a deep-well plate.</li> <li>Add the indicated volume of sample from step 1 on page 28 to the Prepared Lysis Solution.</li> <li>Seal the plate with sealing foil.</li> <li>Shake the plate at moderate speed for 5 minutes.</li> <li>Centrifuge at 3,000 × <i>g</i> for 5 minutes.</li> <li>Remove the supernatant (clarified lysate) without disturbing the pellet.</li> </ol>

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

- 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.
- Transfer the appropriate volume of each clarified lysate (see “Prepare the clarified lysate” on page 28) to a well with the Bead/PK Mix.

Sample type	Volume per well
Oral fluid	600 µL
Environmental samples, fecal samples, and swabs	500 µL

- Mix the sample with the Bead/PK Mix for 2 minutes at room temperature according to your mixing method.
  - Using a plate shaker**—Shake vigorously for 2 minutes (see “Determine the maximum plate shaker setting” on page 10).
  - By pipetting**—Pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL instrument, you must mix by pipetting.)
- Add 350 µL of MagMAX™ CORE Binding Solution.
- Immediately proceed to load the samples into the instrument (next section).

## Process samples on the instrument

1. Select the appropriate script on the instrument (see “Download and install the script” on page 11).
2. Start the run, then load the prepared plates or tube strips in the appropriate positions when prompted by the instrument.

Store purified nucleic acid on ice for immediate use, at  $-20^{\circ}\text{C}$  for up to 1 month, or at  $-80^{\circ}\text{C}$  for long-term storage.

# 7

## Digestion Workflow

The Digestion Workflow is recommended and optimized for the following sample types. The Digestion Workflow is not recommended for purification of RNA.

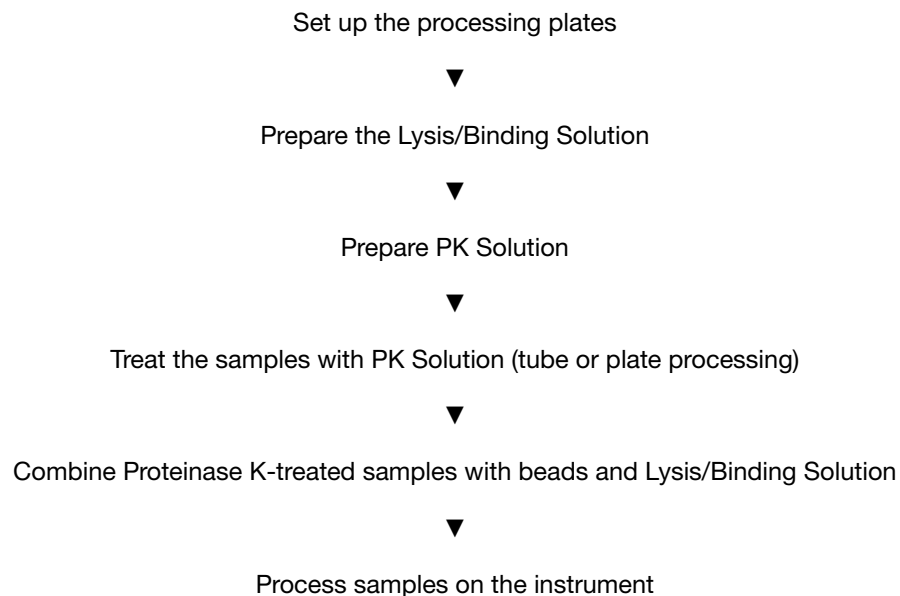
- Environmental samples
- Feces
- Hair follicles
- Swabs—environmental or fecal
- Tissue or organ

Follow this procedure if you are using the KingFisher™ Flex instrument.

If you are using the following instruments, follow the procedure in Appendix B, “Purification with the KingFisher™ Duo Prime or KingFisher™ mL instrument”:

- KingFisher™ Duo Prime
- KingFisher™ mL

### Workflow: Digestion



## Set up the processing plates

1. Set up the processing plates.

**Table 12 Plate setup: KingFisher™ Flex instrument**

Plate ID	Plate position <sup>[1]</sup>	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.	

<sup>[1]</sup> 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
<b>Total Lysis/Binding Solution (-IPC)</b>	<b>700 µL</b>
<i>(Optional)</i> 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
<b>Total Lysis/Binding Solution (+IPC)</b>	<b>700 µL + volume of IPC</b>

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
<b>Total PK Solution</b>	<b>100 µL</b>

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 34.
  - **For plate processing**—proceed to “Treat the samples with PK Solution (plate processing)” on page 36.

## 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	<ol style="list-style-type: none"> <li>Transfer 0.2–0.3 g of sample to a 2-mL tube.</li> <li>Add 1 mL of PBS (1X), pH 7.4, then vortex vigorously for 3 minutes.</li> <li>Centrifuge at <math>100 \times g</math> for 1 minute.</li> <li>Transfer 200 <math>\mu\text{L}</math> of the supernatant to a new tube.</li> <li>Add 100 <math>\mu\text{L}</math> of PK Solution to the transferred supernatant, then vortex briefly to mix.</li> <li>Incubate for 30 minutes at <math>55^\circ\text{C}</math>.</li> <li>Centrifuge at <math>15,000 \times g</math> for 2 minutes.</li> <li>Proceed with 200 <math>\mu\text{L}</math> of digested sample.</li> </ol>
Hair follicles	<ol style="list-style-type: none"> <li>Place 10–15 hair follicles in a 2-mL tube.</li> <li>Add 100 <math>\mu\text{L}</math> of PK Solution to the sample.</li> <li>Incubate for 30 minutes at <math>55^\circ\text{C}</math>.</li> <li>Centrifuge briefly to collect the contents to the bottom of the tube.</li> <li>Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 <math>\mu\text{L}</math>.</li> </ol>
Swabs— environmental or fecal	<ol style="list-style-type: none"> <li><b>Fecal samples</b>—swirl a clinical swab in a fecal sample. <b>Environmental swabs</b>—proceed with an environmental swab.</li> <li>Add 1 mL of PBS (1X), pH 7.4 to a 2-mL tube.</li> <li>Swirl the swab in the PBS (1X), 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 (1X), pH 7.4.</li> <li>Vortex vigorously for 3 minutes, or until the sample is suspended.</li> <li>Centrifuge at <math>100 \times g</math> for 1 minute.</li> <li>Transfer 200 <math>\mu\text{L}</math> of the supernatant to a new tube.</li> <li>Add 100 <math>\mu\text{L}</math> of PK Solution to the transferred supernatant, then vortex briefly to mix.</li> <li>Incubate for 30 minutes at <math>55^\circ\text{C}</math>.</li> <li>Centrifuge at <math>15,000 \times g</math> for 2 minutes.</li> <li>Proceed with 200 <math>\mu\text{L}</math> of digested sample.</li> </ol>
Tissue or organ	<ol style="list-style-type: none"> <li>Transfer 20–30 mg of tissue to a 2-mL tube.</li> <li>Add 100 <math>\mu\text{L}</math> of PK Solution to the sample.</li> <li>Incubate for 2 hours at <math>55^\circ\text{C}</math>.</li> <li>Centrifuge briefly to collect the contents to the bottom of the tube.</li> </ol>

Sample type	Procedure
	<p>e. Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 <math>\mu</math>L. Use a P1000 pipette tip to transfer the viscous sample.</p>

## Treat the samples with PK Solution (plate processing)

Treat samples with PK Solution according to the sample type.

Sample type	Procedure
Environmental samples Feces	<ol style="list-style-type: none"> <li>Transfer 0.2–0.3 g of sample to a well of a 2-mL tube.</li> <li>Add 1 mL of PBS (1X), pH 7.4 to each sample, then vortex vigorously for 3 minutes.</li> <li>Centrifuge at <math>100 \times g</math> for 1 minute.</li> <li>Transfer 200 <math>\mu</math>L of each supernatant to a deep-well plate.</li> <li>Add 100 <math>\mu</math>L of PK Solution to each transferred supernatant, then pipet up and down to mix.</li> <li>Seal the plate with sealing foil.</li> <li>Incubate for 30 minutes at 55°C.</li> <li>Centrifuge at <math>3,000 \times g</math> for 5 minutes.</li> <li>Proceed with 200 <math>\mu</math>L of digested sample.</li> </ol>
Hair follicles	<ol style="list-style-type: none"> <li>Place 10–15 hair follicles in a well of a deep-well plate.</li> <li>Add 100 <math>\mu</math>L of PK Solution to each sample.</li> <li>Seal the plate with sealing foil.</li> <li>Incubate for 30 minutes at 55°C.</li> <li>Centrifuge briefly to collect the contents to the bottom of the plate.</li> <li>Proceed with the volume of digested sample that is available to pipet. The available volume will be less than 100 <math>\mu</math>L.</li> </ol>
Swabs— environmental or fecal	<ol style="list-style-type: none"> <li><b>Fecal samples</b>—swirl a clinical swab in a fecal sample. <b>Environmental swabs</b>—proceed with an environmental swab.</li> <li>Add 1 mL of PBS (1X), pH 7.4 to a 2-mL tube.</li> <li>Swirl the swab in the PBS (1X), 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 (1X), pH 7.4.</li> <li>Vortex vigorously for 3 minutes, or until the samples are suspended.</li> <li>Centrifuge at <math>100 \times g</math> for 1 minute.</li> <li>Transfer 200 <math>\mu</math>L of each supernatant to a deep-well plate.</li> <li>Add 100 <math>\mu</math>L of PK Solution to each transferred supernatant, then pipet up and down to mix.</li> <li>Seal the plate with sealing foil.</li> <li>Incubate for 30 minutes at 55°C.</li> <li>Centrifuge at <math>3,000 \times g</math> for 2 minutes.</li> <li>Proceed with 200 <math>\mu</math>L of digested sample.</li> </ol>
Tissue or organ samples	<ol style="list-style-type: none"> <li>Transfer 20–30 mg of tissue to a well of a deep-well plate.</li> <li>Add 100 <math>\mu</math>L of PK Solution to each sample.</li> <li>Seal the plate with sealing foil.</li> </ol>

Sample type	Procedure
	d. Incubate for 2 hours at 55°C. e. Centrifuge briefly to collect the contents to the bottom of the plate. f. 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.

Sample Type	Volume per well
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 “Determine the maximum plate shaker setting” on page 10).
  - **By pipetting**—Pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL instrument, you must mix by pipetting.)
4. Add 700 µL of Lysis/Binding Solution to each sample.
5. Immediately proceed to load the samples into the instrument (next section).

## Process samples on the instrument

1. Select the appropriate script on the instrument (see “Download and install the script” on page 11).
2. Start the run, then load the prepared plates or tube strips in the appropriate positions when prompted by the instrument.

Store purified nucleic acid on ice for immediate use, at  $-20^{\circ}\text{C}$  for up to 1 month, or at  $-80^{\circ}\text{C}$  for long-term storage.



# Lysis Incubation Workflow

The Lysis Incubation Workflow is recommended and optimized for ear punches that require the following steps:

- An extended lysis step before nucleic acid isolation.
- Addition of punches directly to the MagMAX™ CORE Lysis Solution .

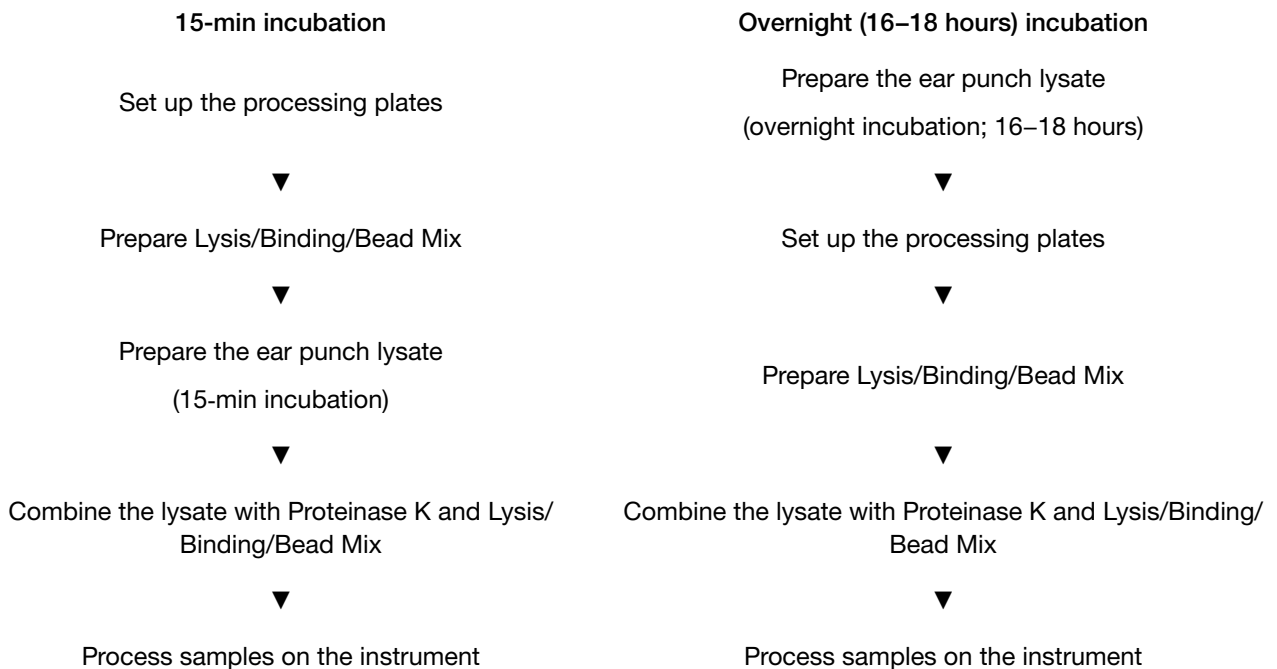
Follow this procedure if you are using the KingFisher™ Flex instrument.

If you are using the following instruments, follow the procedure in Appendix B, “Purification with the KingFisher™ Duo Prime or KingFisher™ mL instrument”:

- KingFisher™ Duo Prime
- KingFisher™ mL

## Workflow: Lysis Incubation

The Lysis Incubation workflow can be performed with a 15-minute 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.



## Set up the processing plates

1. Set up the processing plates.

**Table 13 Plate setup: KingFisher™ Flex instrument**

Plate ID	Plate position <sup>[1]</sup>	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.	

<sup>[1]</sup> 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
<b>Total Lysis/Binding/Bead Mix (-IPC)</b>	<b>720 µL</b>
<i>(Optional)</i> 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
<b>Total Lysis/Binding/Bead Mix (+IPC)</b>	<b>720 µL + volume of IPC</b>

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

## Prepare the ear punch lysate

1. Add 300  $\mu\text{L}$  of MagMAX™ CORE Lysis Solution to each ear punch.
2. Incubate without shaking at room temperature for the desired time.
  - 15 minutes
  - Overnight (16–18 hours)
3. Proceed with individual or pooled supernatants.

Sample type	Action
Individual samples	Proceed with 250 $\mu\text{L}$ of supernatant.
Pooled samples	<ol style="list-style-type: none"> <li>a. Combine 50 <math>\mu\text{L}</math> of individual supernatants in a 2-mL microcentrifuge tube.</li> <li>b. If the volume of pooled supernatants is less than 250 <math>\mu\text{L}</math>, add MagMAX™ CORE Lysis Solution to a total of 250 <math>\mu\text{L}</math>.</li> <li>c. Vortex briefly to mix the pooled samples.</li> <li>d. Proceed with 250 <math>\mu\text{L}</math> of pooled supernatant.</li> </ol> <p>For example:</p> <ul style="list-style-type: none"> <li>• For a pool of 10 samples, the combined volume is 500 <math>\mu\text{L}</math> (10 <math>\times</math> 50 <math>\mu\text{L}</math>). Proceed to the next step with 250 <math>\mu\text{L}</math> of the pool.</li> <li>• For a pool of 4 samples, the combined volume is 200 <math>\mu\text{L}</math> (4 <math>\times</math> 50 <math>\mu\text{L}</math>). Add 50 <math>\mu\text{L}</math> of MagMAX™ CORE Lysis Solution and proceed to the next step with the 250-<math>\mu\text{L}</math> pool.</li> </ul>
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 $\mu\text{L}$ .

For retesting, maintain individual and pooled lysates up to 48 hours at room temperature. To store for longer than 48 hours, store individual and pooled lysates below  $-16^{\circ}\text{C}$ .

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

1. Add 10  $\mu\text{L}$  of MagMAX™ CORE Proteinase K to the required wells in the plate or tube strip.
2. Add 250  $\mu\text{L}$  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  $\mu\text{L}$  of Lysis/Binding/Bead Mix to each sample.
5. Immediately proceed to load the samples into the instrument (next section).

## Process samples on the instrument

1. Select the appropriate script on the instrument (see “Download and install the script” on page 11).
2. Start the run, then load the prepared plates or tube strips in the appropriate positions when prompted by the instrument.

Store purified nucleic acid on ice for immediate use, at  $-20^{\circ}\text{C}$  for up to 1 month, or at  $-80^{\circ}\text{C}$  for long-term storage.



# Troubleshooting

Observation	Possible cause	Recommended action
<p>The eluate is light brown in color</p>	<p>Magnetic beads were carried over into the eluate.</p>	<p>A small quantity of beads in the sample does not inhibit RT-PCR or PCR reactions.</p> <p>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.</p>
<p>Poor or no RNA or DNA signal (that is, the <math>C_t</math> value is higher than expected) In test samples, the <math>C_t</math> value of the IPC target is outside of the validated value range (non-compliant IPC <math>C_t</math> value; invalid sample).</p>	<p>Inhibitors are present in the recovered nucleic acid.</p> <p>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.</p>	<ol style="list-style-type: none"> <li>1. Dilute the invalid nucleic acid sample 1:10 in 1X TE buffer.</li> <li>2. Perform a new PCR analysis with the diluted nucleic acid.</li> </ol> <ul style="list-style-type: none"> <li>• If the diluted nucleic acid is positive for the target, or if it is negative for the target with a compliant IPC <math>C_t</math> value, the result is validated.</li> <li>• If the diluted nucleic acid is negative for the target with a non-compliant IPC <math>C_t</math> value, the result is not validated.</li> </ul> <p>In this case, dilute the original biological sample 1:10 in 1X PBS, then repeat the purification and PCR.</p> <p>If the result is still not validated, then repeat the purification and PCR on a new biological sample.</p>
	<p>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.</p>	<p>Repeat the purification using the Complex workflow.</p> <p>For the samples that show reduced recovery of the IPC RNA or DNA, dilute samples 1:2, 1:4, 1:8, and 1:16 in 1X PBS. Use the dilution that shows the best IPC recovery.</p>

Observation	Possible cause	Recommended action
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 (non-compliant IPC $C_t$ value; invalid sample). <i>(continued)</i>	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 9.
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 $> 20^\circ\text{C}$ . Be sure that you: <ul style="list-style-type: none"> <li>• Vortex the magnetic beads thoroughly before preparing a bead mix.</li> <li>• Fully resuspend the bead mix before adding it to the samples.</li> </ul>
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 determine if 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.
		Avoid splashing when pipetting the reagents or samples.



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

## Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](http://fisherscientific.com) or another major laboratory supplier.

**Table 14** Materials required for processing on the KingFisher™ Duo Prime instrument

Item	Source
KingFisher™ Duo Prime Purification System	<a href="#">5400110</a>
KingFisher™ combi pack for microtiter 96 deep-well plate (includes 8 plates, 8 tip combs, and 8 elution strips and caps) (for Duo Prime only)	<a href="#">97003530</a>
KingFisher™ elution strip for 12 pin magnet (for Duo Prime only) (40 pieces) <sup>[1]</sup>	<a href="#">97003520</a>
KingFisher™ 12-tip comb, for 96 deep-well plate (for Duo Prime only) (50 pieces) <sup>[1]</sup>	<a href="#">97003500</a>
KingFisher™ 96 deep-well plate, sterile (for Duo Prime, Flex and Presto) <sup>[1]</sup>	<a href="#">95040460</a>

<sup>[1]</sup> Included in the KingFisher™ combi pack (Cat. No. [97003530](#)).

**Table 15** Materials required for processing on the KingFisher™ mL instrument

Item	Source
KingFisher™ mL Purification System	<a href="#">5400050</a>
KingFisher™ mL tubes and tip combs (for 240 samples)	<a href="#">97002141</a>
KingFisher™ mL tip comb (800 pieces)	<a href="#">97002111</a>
KingFisher™ mL tubes (20 × 45 pieces)	<a href="#">97002121</a>

## 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 16 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 <sup>[1]</sup>	Separate tube strip <sup>[2]</sup>	Elution strip	MagMAX™ CORE Elution Buffer	90 µL
Tip Comb	H	Deep Well	Place a tip comb in the plate.	

<sup>[1]</sup> Ensure that the elution strip is placed in the correct direction in the elution block.

<sup>[2]</sup> Placed on the heating element.

**Table 17 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 17.



# 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, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).



## 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.



**WARNING! HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



**WARNING! 4L Reagent and Waste Bottle Safety.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.



## Biological hazard safety



**WARNING! Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



**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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020  
[cdc.gov/labs/bmbi](https://www.cdc.gov/labs/bmbi)
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)  
[who.int/publications/i/item/9789240011311](https://www.who.int/publications/i/item/9789240011311)



# Documentation and support

## Related documentation

Document	Publication Number
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	N07669
<i>Thermo Scientific™ KingFisher™ Duo Prime Technical Manual</i>	N16621
<i>Thermo Scientific™ KingFisher™ mL User Manual</i>	1508260
<i>MagMAX™ CORE Mechanical Lysis Module User Guide</i>	MAN0015945

## Customer and technical support

Visit [thermofisher.com/support](http://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)

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

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## Limited product warranty

Life Technologies Corporation and its affiliates 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](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have questions, contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).

