

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 D00



Revision history: MAN0015944 D00 (English)

| Revision | Date | Description |
|----------|------------------|---|
| D.00 | 25 April 2024 | <ul style="list-style-type: none">• New alternate workflows for whole blood and semen samples were added (see Chapter 4, “Whole Blood Workflow” and Chapter 5, “Semen Workflow”).• The MagMAX™ Express-96 was removed due to product discontinuation.• Minor edits were made for style and consistency. |
| C.0 | 13 December 2017 | <ul style="list-style-type: none">• Updated the usage statement on the front cover.• Minor reorganization of the required materials section for style and clarity.• Minor corrections to product names. Minor edits for style and consistency. |
| B.0 | 30 June 2017 | <ul style="list-style-type: none">• Combined and renamed workflows:<ul style="list-style-type: none">– 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 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

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

^[1] 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^[1]

| Item | Source |
|---|---|
| Instrument and equipment | |
| KingFisher™ Flex Purification System See page 46 for other compatible instruments. | Contact your local sales office. |
| Benchtop microcentrifuge capable of 15,000 × g | MLS |
| Laboratory mixer, Vortex or equivalent | MLS |
| Reagents | |
| PBS (1X), pH 7.4 ^[2] | 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 | |
| 5-mL tubes, or equivalent | MLS |
| 2-mL tubes, or equivalent | MLS |
| Adhesive PCR Plate Foils, or equivalent | AB0626 |
| KingFisher™ Flex Microtiter Deep-Well 96 plates, 50 plates | 95040460 |
| KingFisher™ 96 KF microplates (200 µL), 48 plates | 97002540 |
| KingFisher™ 96 tip comb for deep-well magnets, 100 combs | 97002534 |

^[1] See Table 4 and Table 5 for additional materials required for the Simple and Digestion workflows.

^[2] Not required for the Lysis Incubation workflow.

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™ 15-340-163 |
| PYREX™ Solid Glass Beads for Distillation Columns (3 mm) | Fisher Scientific™ 11-312-10A |

Table 5 Additional materials required for the Digestion and alternate Whole Blood and Semen workflows

| Item | Source |
|---|-------------------------|
| Laboratory benchtop incubator, or equivalent | MLS |
| PK Buffer for MagMAX™-96 DNA Multi-Sample Kit | 4489111 |
| 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"> Ear punch (circular shape, 2- to 3-mm diameter) in Lysis Solution | Viral nucleic acid | Lysis Incubation ^[1] (page 39) |
| <ul style="list-style-type: none"> Ear punch (circular shape, 2- to 3-mm diameter) in PBS Ear notch (triangular shape, approximately 1-cm width) Milk Plasma Serum | <ul style="list-style-type: none"> Viral nucleic acid Bacterial DNA | Simple (page 12) |
| <ul style="list-style-type: none"> Biomed Diagnostics InPouch™ TF (<i>Tritrichomonas foetus</i>) culture | <i>Tritrichomonas foetus</i> DNA | |
| <ul style="list-style-type: none"> Semen^[2] | Viral nucleic acid | |
| <ul style="list-style-type: none"> Swabs—animal Whole blood^[3] | <ul style="list-style-type: none"> Viral nucleic acid Genomic DNA | |
| <ul style="list-style-type: none"> Tissue or organ | <ul style="list-style-type: none"> Viral nucleic acid Bacterial DNA^[4] | |
| <ul style="list-style-type: none"> Hair follicles | Genomic DNA | Digestion (page 31) |
| <ul style="list-style-type: none"> Environmental samples Feces Swabs—environmental or fecal | Bacterial DNA <ul style="list-style-type: none"> Viral nucleic acid Bacterial DNA^[4] | Complex (page 25) |
| <ul style="list-style-type: none"> Oral fluid | <ul style="list-style-type: none"> Viral nucleic acid Bacterial DNA | |
| <ul style="list-style-type: none"> Semen | Viral nucleic acid | Semen (page 21) |
| <ul style="list-style-type: none"> Whole blood | <ul style="list-style-type: none"> Viral nucleic acid Genomic DNA | Whole Blood (page 18) |

^[1] Recommended if overnight incubation is required.

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

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

^[4] 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 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 tube strips that are required by the KingFisher™ mL instrument.

- To prevent cross-contamination:
 - Cover the plate or tube strip during the incubation and shaking steps, to prevent spill-over.
 - Carefully pipet reagents and samples, to avoid splashing.
- To prevent nuclease contamination:
 - Wear laboratory gloves during the procedures. Gloves protect you from the reagents, and they protect the nucleic acid from nucleases that are present on skin.
 - Use nucleic acid-free pipette tips to handle the reagents, and avoid putting used tips into the reagent containers.
 - Decontaminate lab benches and pipettes before you begin.

Before first use of the kit

Determine the maximum plate shaker setting

If a plate shaker is used, 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, 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.



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

IMPORTANT!

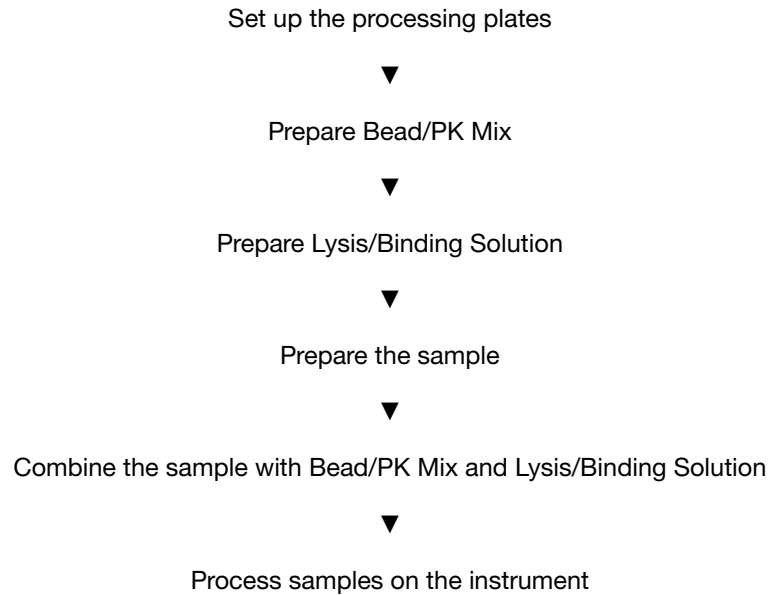
- If you have encountered bead coagulation or aggregation with whole blood samples, follow the Whole Blood Workflow (page 18).
 - If you have encountered low nucleic acid recovery, follow the Semen Workflow (page 21).
-

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

If you are using the following instruments, follow 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 ^[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 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 |
| Total Bead/PK Mix | 30 µL |

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 |
| Total Lysis/Binding Solution (-IPC) | 700 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 |
| Total Lysis/Binding Solution (+IPC) | 700 µL + volume of IPC |

- 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"> Add one ear notch to a 5-mL specimen tube. Add 2 mL of PBS (1X), pH 7.4 to each sample. Incubate at room temperature with or without shaking: <ul style="list-style-type: none"> Without shaking—15 minutes With moderate shaking—10 minutes Proceed with 200 µL of supernatant. |
| Ear punch (circular shape, 2- to 3-mm diameter) | <ol style="list-style-type: none"> Add one ear punch to a 2-mL tube. Add 200 µL of PBS (1X), pH 7.4 to each sample. Incubate at room temperature with or without shaking: <ul style="list-style-type: none"> Without shaking—15 minutes With moderate shaking—10 minutes Proceed with 50–200 µL of supernatant. |
| Milk, plasma, serum, or whole blood ^[1] | Proceed with 200 µL of sample. |

(continued)

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

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

^[2] 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 |
| Total Lysis/Binding Solution (–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 Solution (+IPC) | 720 µL + volume of IPC |

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.

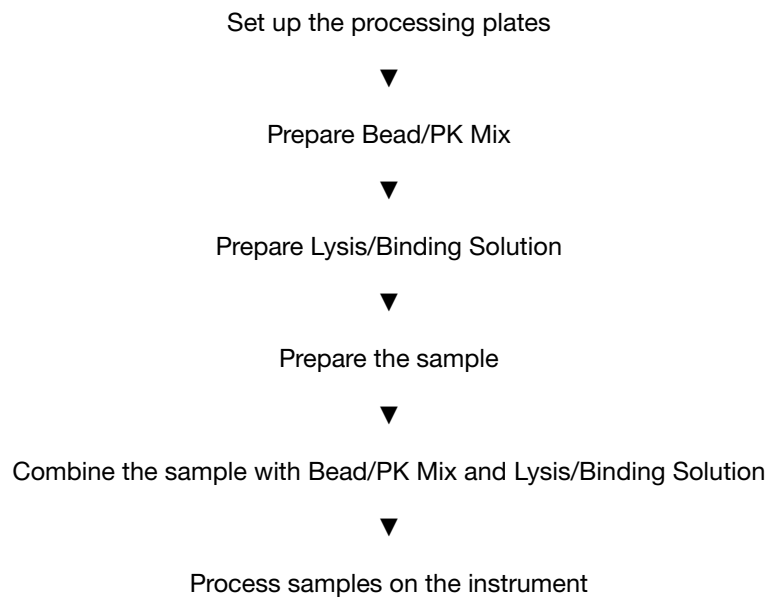
Store purified nucleic acid on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

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 ^[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 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 |
| Total PK/PBS Mix | 200 µL |

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 |
| 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 |

(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 |
| Total Lysis/Binding Mix (+IPC) | 700 µL + volume of IPC |

- 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. |
| NCS | — |

Combine the sample with PK/PBS Mix and Lysis/Binding 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.

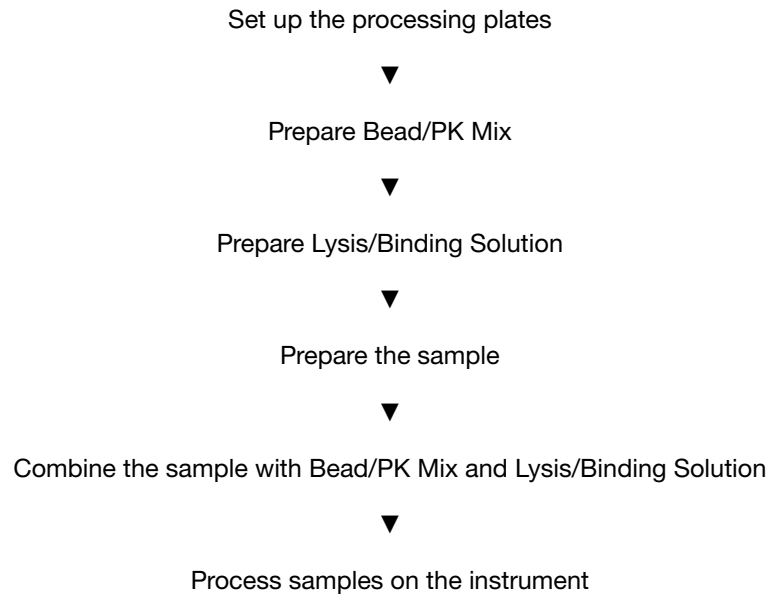
Store purified nucleic acid on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.



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 ^[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 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 |
| Total PK/PBS Mix | 210 µL |

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 |
| Total Binding/Bead Mix (–IPC) | 420 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 |

(continued)

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

- 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 |
|-------------|--------------------------------|
| Whole blood | Proceed with 300 µL of sample. |
| NCS | — |

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

- Vortex the tube of Lysis Solution, then add 400 µL of the Lysis Solution to the required wells in the plate or tube strip.
- Transfer 300 µL of each semen sample to a well with 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

- 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.



Complex Workflow

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

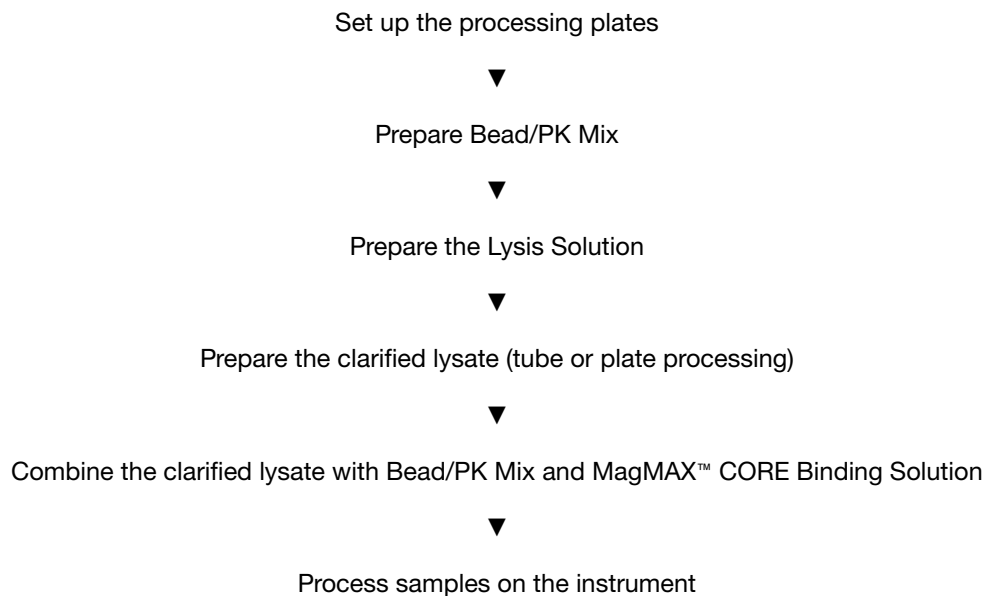
- Environmental samples
- Feces
- Oral fluid
- Swabs—environmental or fecal

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

If you are using the following instruments, follow 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 ^[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 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 |
| 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 |
| <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 |
| 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.

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

2. Add Lysis Solution, then clarify the lysate.

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

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°C for up to 1 month, or at -80°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.

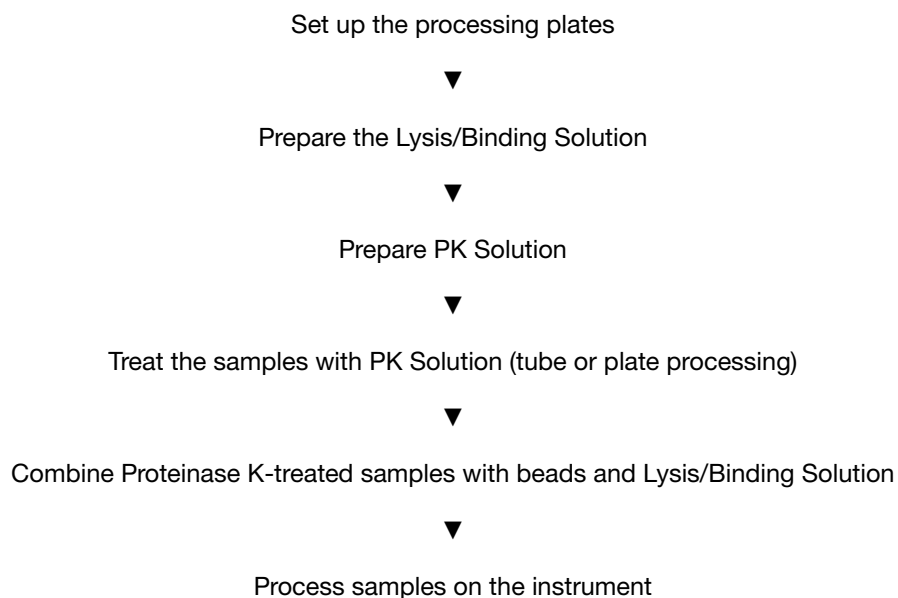
- Environmental samples
- Feces
- Hair follicles
- Swabs—environmental or fecal
- Tissue or organ

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

If you are using the following instruments, follow 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 ^[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 |
| <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 |
| 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 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"> Transfer 0.2–0.3 g of sample to a 2-mL tube. Add 1 mL of PBS (1X), pH 7.4, then vortex vigorously for 3 minutes. Centrifuge at $100 \times g$ for 1 minute. Transfer 200 μL of the supernatant to a new tube. Add 100 μL of PK Solution to the transferred supernatant, then vortex briefly to mix. Incubate for 30 minutes at 55°C. Centrifuge at $15,000 \times g$ for 2 minutes. Proceed with 200 μL of digested sample. |
| Hair follicles | <ol style="list-style-type: none"> Place 10–15 hair follicles in a 2-mL tube. Add 100 μL of PK Solution to the sample. Incubate for 30 minutes at 55°C. Centrifuge briefly to collect the contents to the bottom of the tube. 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 | <ol style="list-style-type: none"> Fecal samples—swirl a clinical swab in a fecal sample. Environmental swabs—proceed with an environmental swab. Add 1 mL of PBS (1X), pH 7.4 to a 2-mL tube. 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. Vortex vigorously for 3 minutes, or until the sample is suspended. Centrifuge at $100 \times g$ for 1 minute. Transfer 200 μL of the supernatant to a new tube. Add 100 μL of PK Solution to the transferred supernatant, then vortex briefly to mix. Incubate for 30 minutes at 55°C. Centrifuge at $15,000 \times g$ for 2 minutes. Proceed with 200 μL of digested sample. |
| Tissue or organ | <ol style="list-style-type: none"> Transfer 20–30 mg of tissue to a 2-mL tube. Add 100 μL of PK Solution to the sample. Incubate for 2 hours at 55°C. Centrifuge briefly to collect the contents to the bottom of the tube. |

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

| 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°C for up to 1 month, or at -80°C for long-term storage.



Lysis Incubation Workflow

The Lysis Incubation Workflow is recommended and optimized for ear punches that require processing with:

- An extended lysis step before nucleic acid isolation.
- Addition of punches directly to a lysis solution.

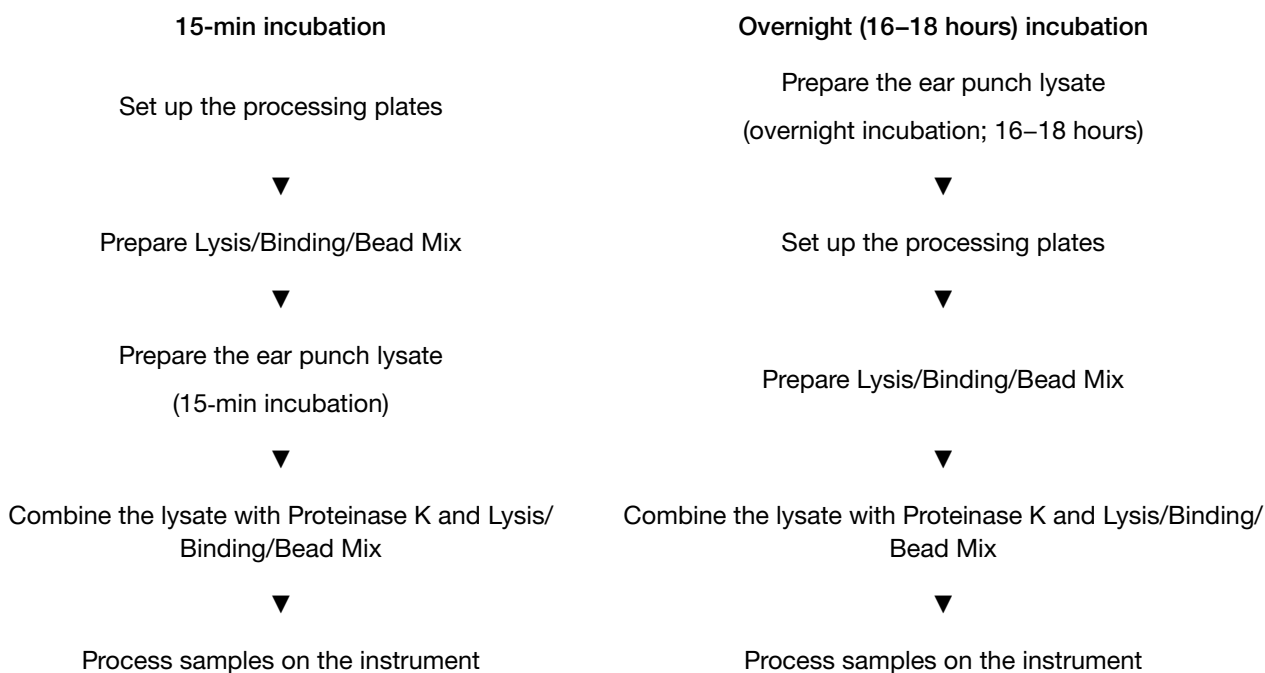
Follow this procedure if you are using the KingFisher™ Flex instrument.

If you are using the following instruments, follow 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 ^[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 |
| <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 |
| 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.
 - 15 minutes
 - Overnight (16–18 hours)
3. Proceed with individual or pooled supernatants.

| Sample type | Action |
|--|---|
| Individual samples | Proceed with 250 μL of supernatant. |
| Pooled samples | <ol style="list-style-type: none"> a. Combine 50 μL of individual supernatants in a 2-mL microcentrifuge tube. b. If the volume of pooled supernatants is less than 250 μL, add MagMAX™ CORE Lysis Solution to a total of 250 μL. c. Vortex briefly to mix the pooled samples. d. Proceed with 250 μL of pooled supernatant. <p>For example:</p> <ul style="list-style-type: none"> • For a pool of 10 samples, the combined volume is 500 μL (10 \times 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 \times 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 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°C for up to 1 month, or at -80°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 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).</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"> 1. Dilute the invalid nucleic acid sample 1:10 in 1X TE buffer. 2. Perform a new PCR analysis with the diluted nucleic acid. <ul style="list-style-type: none"> • If the diluted nucleic acid is positive for the target, or if it is negative for the target with a compliant IPC C_t value, the result is validated. • If the diluted nucleic acid is negative for the target with a non-compliant IPC C_t value, the result is not validated. <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 |
|---|--|---|
| <p>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></p> | <p>The IPC DNA or RNA did not bind efficiently to the magnetic beads, due to extracellular material in the sample.</p> | <p>Add MagMAX™ CORE Magnetic Beads to the Lysis/Binding Solution, instead of preparing Bead/PK Mix or adding beads directly to the sample.</p> |
| <p>Poor yield of viral RNA from tissue, fecal or environmental samples, or swabs</p> | <p>The Digestion workflow was used for viral nucleic acid purification.</p> | <p>Follow the appropriate workflow. See “Recommended workflows” on page 9.</p> |
| <p>Well-to-well variation in RNA/DNA yield from replicate samples</p> | <p>The magnetic beads were not fully resuspended/dispersed.</p> | <p>In general, the magnetic beads disperse more easily when the temperature of the mixture is > 20°C. Be sure that you:</p> <ul style="list-style-type: none"> • Vortex the magnetic beads thoroughly before preparing a bead mix. • Fully resuspend the bead mix before adding it to the samples. |
| <p>Positive samples are clustered in the PCR plate</p> | <p>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.</p> | <p>Repeat the nucleic acid purification of the positive or suspect samples without the high-titer samples.</p> |
| | | <p>Avoid splashing when pipetting the reagents or samples.</p> |



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" indicates that the material is available from 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 | 5400110 |
| 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) | 97003530 |
| KingFisher™ elution strip for 12 pin magnet (for Duo Prime only) (40 pieces) ^[1] | 97003520 |
| KingFisher™ 12-tip comb, for 96 deep-well plate (for Duo Prime only) (50 pieces) ^[1] | 97003500 |
| KingFisher™ 96 deep-well plate, sterile (for Duo Prime, Flex and Presto) ^[1] | 95040460 |

^[1] 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 | 5400050 |
| KingFisher™ mL tubes and tip combs (for 240 samples) | 97002141 |
| KingFisher™ mL tip comb (800 pieces) | 97002111 |
| KingFisher™ mL tubes (20 × 45 pieces) | 97002121 |

Purification procedure

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

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

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

2. Add MagMAX™ CORE Wash Solutions and MagMAX™ CORE Elution Buffer to the indicated positions, according to your instrument.
Load the Tip Comb and all of the plates or tube strips at the same time. The instrument does not prompt you to load items individually.

Table 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 ^[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 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
www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
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 |

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

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