VetMAX™ C. burnetii Feces Kit

Nucleic acid purification protocols optimized for use with the kit (Cat. No. FQPE)

Pub. No. MAN0019383 Rev. B.0

Species	Sample matrices	Test type
• Bovine • Small ruminants (sheep, goats)	• Feces • Wipes and boot swabs	Individual

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

WARNING! BIOHAZARD. Read the biological hazard safety information at this product's page at thermofisher.com. Follow all applicable local, state/provincial, and/or national regulations for working with biological samples.

Purpose of this guide

This guide describes *Coxiella burnetii* bacterial DNA purification protocols that have been validated and optimized for downstream use with the Applied Biosystems™ VetMAX™ C. burnetii Feces Kit (Cat. No. FQPE).

- Automated nucleic acid purification is performed using one of the following instruments: KingFisher™ Flex, MagMAX™ Express-96, KingFisher™ mL, or KingFisher™ Duo Prime.
- Manual nucleic acid purification uses silica-based spin columns or plates.

Sample selection

Sample type	Type of analysis	Quantity required
Feces	Individual	1 mL of liquid feces or 1 g of solid feces[1]
Wipes and boot swabs	Individual	200 µL of eluate

[1] Depending on the purification protocol used.

Sample storage

Sample type	Storage
Feces	After collection, maintain the samples at 2°C to 8°C until use (up to 2 days). After use or after 2 days, store samples below –16°C for up to 1 year, or below –70°C for long-term storage.
Wipes and boot swabs	After collection, place the wipe or swab into a sterile bag, then decontaminate the outside of the bag with a diluted bleach solution. Maintain the samples at 2°C to 8°C until use (up to 2 days). After use or after 2 days, store samples below –16°C for up to 1 year, or below –70°C for long-term storage.

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.

Catalog numbers that appear as links open the web pages for those products.

Materials required for sample collection, preparation, and nucleic acid purification

Table 1 Materials required for all sample preparation methods

Item	Source
Equipment	
Type II Biological Safety Cabinet (BSCII)	MLS
Benchtop microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Adjustable precision micropipettors (range of 1 µL to 1,000 µL)	MLS
Precision scale	MLS
Consumables	
Aerosol-resistant, nuclease-free pipette tips	MLS
1.5-mL and 2.0-mL DNase/RNase-free microtubes	MLS
10-mL tubes	MLS
Reagents	
5 – IPC Cox b. FE	From the VetMAX™ C. burnetii Feces Kit (Cat. No. FQPE)
4d – EPC Cox b. FE	
Nuclease-free water	AM9932
PBS (1X), pH 7.4	MLS

Additional materials required for automated nucleic acid purification

Table 2 Materials required for the MagMAX™ CORE Nucleic Acid Purification Kit

Item	Source
Instrument, one of the following:	
KingFisher™ Flex Purification System	Contact your local sales office.
MagMAX™ Express-96 Magnetic Particle Processor	
KingFisher™ Duo Prime Purification System	
KingFisher™ mL Purification System	
Equipment	
Heat block at 55°C	MLS
Reagent reservoir	MLS
PYREX™ Solid Glass Beads for Distillation Columns (5 mm), or equivalent 5-mm glass beads	Fisher Scientific™ 11-312-10C
Consumables	
Adhesive PCR Plate Foils, or equivalent	AB0626
Consumables for the KingFisher™ Flex and MagMAX™ Express-96 instruments: • KingFisher™ 96 Deep-Well Plate • KingFisher™ 96 KF microplates • KingFisher™ 96 tip comb for deep-well magnets	 • 95040450 • 97002540 • 97002534
Consumables for the KingFisher™ Duo Prime and KingFisher™ mL instruments	See Table 8 on page 15.
Kits and reagents	
MagMAX™ CORE Nucleic Acid Purification Kit	A32700 or A32702
PBS, pH 7.4 (10X), RNase-free	AM9624
PK Buffer for MagMAX™-96 DNA Multi-Sample Kit	4489111
UltraPure™ SDS Solution, 10%	24730020

Additional materials required for manual nucleic acid purification

Table 3 Materials required for all manual methods (individual, plate, and tube-strip formats)

Item	Source
Equipment	
Heat block at 70°C	MLS
Kits and reagents	
One of the following kits: • QIAamp™ DNA Mini Kit • NucleoSpin™ Tissue kit • QIAamp™ 96 DNA Swab BioRobot Kit • NucleoSpin™ 96 Tissue kit • NucleoSpin™ 8 Tissue kit	 • Qiagen 51304 • Macherey Nagel 740952 • Qiagen 965842 • Macherey Nagel 740741.4 • Macherey Nagel 740740
Ethanol, 96–100%	MLS

Table 4 Materials required for manual purification in plate or tube-strip format

Item	Source
Plate centrifuge	MLS
Adhesive film	MLS

Recommended DNA purification protocols

Sample type	Automated purification	Manual purification
Feces	MagMAX™ CORE Nucleic Acid Purification Kit (page 4)	• QIAamp™ DNA Mini Kit (page 9) • NucleoSpin™ Tissue kit (page 11) • QIAamp™ 96 DNA Swab BioRobot Kit (page 12) • NucleoSpin™ 8 Tissue/NucleoSpin™ 96 Tissue kit (page 13)
Wipes and boot swabs	—	• QIAamp™ DNA Mini Kit (page 9) • NucleoSpin™ Tissue kit (page 11)

Procedural guidelines

Process the following controls concurrently with the test samples, using the same purification protocol:

- Mock-purified sample for use as a negative extraction control—Use PBS (1X), pH 7.4, or nuclease-free water in place of the test sample, unless otherwise directed.
- External positive control (EPC)—Use 4d – EPC Cox b. FE that is supplied with the VetMAX™ C. burnetii Feces Kit. Purified EPC can be stored for use in subsequent real-time PCR assays with samples that are purified using the same protocol.

Purify nucleic acid using the MagMAX™ CORE Nucleic Acid Purification Kit (automated method)

This procedure is designed for rapid purification of bacterial DNA from feces.

Follow this procedure if you are using these instruments:

- KingFisher™ Flex
- MagMAX™ Express-96

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

- KingFisher™ Duo Prime
- KingFisher™ mL

MagMAX™ CORE Nucleic Acid Purification Kit workflow

Set up the processing plates

Prepare Lysis/Binding/Bead/IPC Solution

Prepare PK Solution

Prepare the sample

Treat samples with the PK Solution

Combine PK-treated samples with the Lysis/Binding/Bead/IPC Solution

Process samples on the instrument

Procedural guidelines

- Before use, invert bottles of solutions and buffers to ensure thorough mixing.
- 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.

Download and install the script

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

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

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

   Table 5 Recommended scripts

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

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

   Table 6 Alternate scripts without heated elution step

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

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

Perform the purification procedure

1 Set up the processing plates

1.1. Set up the processing plates.

Table 7 Plate setup: KingFisher™ Flex or MagMAX™ Express-96 instrument

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

[1] Position on the instrument.

Note: To set up processing plates or tube strips for the KingFisher™ Duo Prime or KingFisher™ mL instrument, see Appendix A, "Purification with the KingFisher™ Duo Prime or KingFisher™ mL instrument".

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

2 Prepare Lysis/Binding/Bead/IPC Solution

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

2.2. Combine the following components for the required number of samples, plus up to 10% overage.

Component	Volume per sample
MagMAX™ CORE Lysis Solution	350 µL
MagMAX™ CORE Binding Solution	350 µL
MagMAX™ CORE Magnetic Beads	20 µL
5 – IPC Cox b. FE	5 µL
Total Lysis/Binding/Bead/IPC Solution	**725 µL**

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

3 Prepare PK Solution

Prepare PK Solution immediately before use.

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

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**

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

3.3. Proceed immediately to the next step.

4 Prepare the sample

4.1. Add the following components to a 10-mL conical tube.

- Feces—1 ± 0.1 mL of liquid sample or 1 ± 0.1 g of solid sample
- SDS (1%)—4 mL
- PYREX™ Solid Glass Beads for Distillation Columns (5 mm)—3 beads

4.2. Vortex vigorously for 5 minutes.

4.3. Centrifuge at 1,500 × *g* for 1 minute.

Alternatively, incubate the samples on a surface that is free of vibration for 5 minutes.

4.4. Immediately proceed to the next step with 200 µL of supernatant.

5 Treat samples with the PK Solution

5.1. Transfer the appropriate volume of each sample to a new tube or well of a Deep Well plate.

For...	Use...
Feces	200 µL of supernatant
Mock-purified sample	200 µL of PBS (1X), pH 7.4, or nothing
Positive control	200 µL of 4d – EPC Cox b. FE

5 Treat samples with the PK Solution *(continued)*

5.2. Treat the sample with the PK Solution.

For...	Do this...
Tube processing	1. Add 100 µL of the PK Solution to each sample, then vortex briefly to mix. 2. Incubate for 30 minutes at 55°C. 3. Centrifuge briefly to collect contents at the bottom of the tube. 4. Proceed with 300 µL of PK-treated sample.
Plate processing	1. Add 100 µL of the PK Solution to each sample, then pipet up and down to mix. 2. Seal the plate with sealing foil. 3. Incubate for 30 minutes at 55°C. 4. Centrifuge briefly to collect contents at the bottom of the tube. 5. Proceed with 300 µL of PK-treated sample.

6 Combine PK-treated samples with the Lysis/Binding/Bead/IPC Solution

6.1. Add 300 µL of each PK-treated sample to the appropriate wells in the sample plate or tube strip.

6.2. Vortex the Lysis/Binding/Bead/IPC Solution thoroughly to ensure that the beads are fully resuspended.

6.3. Add 725 µL of Lysis/Binding/Bead/IPC Solution to each sample.

6.4. Immediately proceed to process samples on the instrument (next section).

7 Process samples on the instrument

7.1. Select the appropriate script on the instrument (see “Download and install the script” on page 6).

7.2. Start the run, then load the prepared plates 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.

Prepare samples for purification with other kits

Prepare samples as described.

Sample type	Purification method	Action
Feces	Automated	1. Add the following components to a 50-mL conical tube. • Feces—1 g of solid sample • PBS (1X), pH 7.4—10 mL • PYREX™ Solid Glass Beads for Distillation Columns (3 mm)—3 beads 2. Vortex for 1 minute. 3. Immediately transfer 1 mL of the suspension to a 1.5-mL tube. 4. Centrifuge at 1,000 × *g* for 2 minutes. 5. Proceed with 200 µL of supernatant.
	Manual	1. Add the following components to a 10-mL conical tube. • Feces—1 g of solid sample • PBS (1X), pH 7.4—4 mL 2. Vortex for 1 minute. 3. Immediately transfer 400 µL of the suspension to a 1.5-mL tube. 4. Centrifuge at 6,000 × *g* for 1 minute. 5. Proceed with 200 µL of supernatant.
Wipes or boot swabs	Manual	1. Add 40 mL of PBS (1X), pH 7.4 to the bag containing the dry wipe or boot swab. 2. Knead the bag for 1 minute. 3. Transfer 400 µL of the suspension to a 1.5-mL tube. 4. Centrifuge at 6,000 × *g* for 1 minute. 5. Proceed with 200 µL of supernatant.

Purify DNA using the QIAamp™ DNA Mini Kit (manual method)

Before first use of the kit

Reconstitute the AW1 and AW2 Buffers—Add the required volume of 96–100% ethanol according to the recommendations of the supplier.

Perform the purification procedure

1 Lyse, then homogenize the samples (feces)

1.1. Combine the following components in the order indicated, then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock-purified sample	Volume per positive control
Prepared sample	200 µL of supernatant	—	—
PBS (1X), pH 7.4	—	200 µL	—
4d – EPC Cox b. FE	—	—	200 µL
5 – IPC Cox b. FE	5 µL	5 µL	5 µL
AL Buffer	200 µL	200 µL	200 µL
Proteinase K	20 µL	20 µL	20 µL

1.2. Vortex for 15 seconds.

1.3. Incubate at 70°C for 30 minutes.

1.4. Allow the tubes to cool, then centrifuge the samples briefly to bring down condensation.

1.5. Add 200 µL of 96–100% ethanol to each sample, vortex for 15 seconds, then briefly centrifuge to collect the contents.

2 Lyse, then homogenize the samples (wipes or boot swabs)

2.1. Combine the following components in the order indicated, then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock-purified sample	Volume per positive control
Prepared sample	200 µL of supernatant	—	—
PBS (1X), pH 7.4	—	200 µL	—
4d – EPC Cox b. FE	—	—	200 µL
5 – IPC Cox b. FE	5 µL	5 µL	5 µL
ATL Buffer	180 µL	180 µL	180 µL
Proteinase K	20 µL	20 µL	20 µL

2.2. Vortex for 15 seconds.

2.3. Incubate at 70°C for 30 minutes.

2.4. Allow the tubes to cool, then centrifuge the samples briefly to bring down condensation.

2.5. Add 200 µL of AL Buffer, then vortex for 15 seconds.

2.6. Add 200 µL of 96–100% ethanol to each sample, vortex for 15 seconds, then briefly centrifuge to collect the contents.

3 Bind the DNA to the column

3.1. Insert a QIAamp™ DNA Mini Kit column into a collection tube, then transfer the entire sample volume to the column.

3.2. Cap the column, then centrifuge the assembly at 15,000 × *g* for 1 minute.

3.3. Discard the collection tube, then place the column on a new collection tube.

4 Wash, then elute the DNA

4.1. Add 500 µL of AW1 Buffer to each column, cap the column, then centrifuge at 15,000 × *g* for 1 minute.

4.2. Discard the collection tube, then place the column on a new collection tube.

4.3. Add 500 µL of AW2 Buffer to each column, cap the column, then centrifuge at 15,000 × *g* for 1 minute.

4.4. Discard the collection tube, then place the column on a new collection tube.

4.5. Centrifuge at 15,000 × *g* for 3 minutes to dry the membrane.

4.6. Discard the collection tube.

4.7. Place the column on a new 1.5-mL microtube, then add 200 µL of AE Buffer.

4.8. Cap the column, then incubate at room temperature for 1 minute.

4.9. Centrifuge at 6,000 × *g* for 1 minute, then discard the column.

The purified DNA is in the microtube.

Store the purified DNA at 2–8°C for immediate use or below –16°C for long-term storage.

Purify DNA using the NucleoSpin™ Tissue kit (manual method)

Before first use of the kit

- Reconstitute the B5 Buffer—Add the required volume of 96–100% ethanol according to the recommendations of the supplier.
- Reconstitute the PK—Add the required volume of PB Buffer according to the recommendations of the supplier.

Perform the purification procedure

1 Lyse, then homogenize the samples (feces)

1.1. Combine the following components in the order indicated, then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock-purified sample	Volume per positive control
Prepared sample	200 µL of supernatant	—	—
PBS (1X), pH 7.4	—	200 µL	—
4d – EPC Cox b. FE	—	—	200 µL
5 – IPC Cox b. FE	5 µL	5 µL	5 µL
B3 Buffer	200 µL	200 µL	200 µL
Proteinase K	25 µL	25 µL	25 µL

1.2. Vortex for 15 seconds.

1.3. Incubate at 70°C for 30 minutes.

1.4. Allow the tubes to cool, then centrifuge the samples briefly to bring down condensation.

1.5. Add 200 µL of 96–100% ethanol to each sample, vortex for 15 seconds, then briefly centrifuge to collect the contents.

2 Lyse, then homogenize the samples (wipes or boot swabs)

2.1. Combine the following components in the order indicated, then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock-purified sample	Volume per positive control
Prepared sample	200 µL of supernatant	—	—
PBS (1X), pH 7.4	—	200 µL	—
4d – EPC Cox b. FE	—	—	200 µL
5 – IPC Cox b. FE	5 µL	5 µL	5 µL
T1 Buffer	180 µL	180 µL	180 µL
Proteinase K	25 µL	25 µL	25 µL

2.2. Add 200 µL of B3 Buffer, then vortex for 15 seconds.

2.3. Incubate at 70°C for 10 minutes.

2.4. Allow the tubes to cool, then centrifuge the samples briefly to bring down condensation.

2.5. Add 200 µL of 96–100% ethanol to each sample, vortex for 15 seconds, then briefly centrifuge to collect the contents.

3 Bind the DNA to the column

3.1. Insert a NucleoSpin™ Tissue kit column into a collection tube, then transfer the entire sample volume to the column.

3.2. Cap the column, then centrifuge the assembly at 11,000 × *g* for 1 minute.

3.3. Discard the collection tube, then place the column on a new collection tube.

4 Wash, then elute the DNA

4.1. Add 500 µL of BW Buffer to each column, cap the column, then centrifuge at 11,000 × *g* for 1 minute.

4.2. Discard the collection tube, then place the column on a new collection tube.

4.3. Add 600 µL of B5 Buffer to each column, cap the column, then centrifuge at 11,000 × *g* for 1 minute

4.4. Discard the collection tube, then place the column on a new collection tube.

4.5. Centrifuge at 11,000 × *g* for 3 minutes to dry the membrane.

4.6. Discard the collection tube.

4.7. Place the column on a new 1.5-mL microtube, then add 200 µL of BE Buffer.

4.8. Cap the column, then incubate at room temperature for 1 minute.

4.9. Centrifuge at 11,000 × *g* for 1 minute, then discard the column.

The purified DNA is in the microtube.

Store the purified DNA at 2–8°C for immediate use or below –16°C for long-term storage.

Purify DNA using the QIAamp™ 96 DNA Swab BioRobot Kit (manual method)

Before first use of the kit

Reconstitute the AW1 and AW2 Buffers—Add the required volume of 96–100% ethanol according to the recommendations of the supplier.

Perform the purification procedure

1 Lyse, then homogenize the samples

1.1. Combine the following components in a lysis plate (S-Block 1), then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock-purified sample	Volume per positive control
Prepared sample	200 µL of supernatant	—	—
PBS (1X), pH 7.4	—	200 µL	—
4d – EPC Cox b. FE	—	—	200 µL
5 – IPC Cox b. FE	10 µL	10 µL	10 µL
AL Buffer	200 µL	200 µL	200 µL
Proteinase K	20 µL	20 µL	20 µL

1.2. Pipet up and down 5 times to mix, then seal the plate with adhesive film.

1.3. Incubate at 70°C for 30 minutes or at 56°C for 16–18 hours.

1.4. Allow the samples to cool, then centrifuge the samples briefly to bring down condensation.

1.5. Add 200 µL of 96–100% ethanol to each sample, then mix by pipetting up and down 5 times.

2 Bind the DNA to the column

2.1. Place a QIAamp™-96 plate (extraction plate) on a new S-Block, then transfer each lysate from S-Block 1 to the appropriate wells of the extraction plate.

2.2. Seal the plate with adhesive film, then centrifuge the plate/S-Block assembly at 6,000 × *g* for 4 minutes.

2.3. Discard the S-Block, then place the extraction plate on a new S-Block.

3 Wash, then elute the DNA

3.1. Add 500 µL of AW1 Buffer to each well, seal with adhesive film, then centrifuge at 6,000 × *g* for 2 minutes.

3.2. Discard the S-Block, then place the extraction plate on a new S-Block.

3.3. Add 500 µL of AW2 Buffer to each well, seal with adhesive film, then centrifuge at 6,000 × *g* for 2 minutes.

3.4. Discard the S-Block, then place the extraction plate on a new S-Block.

3.5. Remove the adhesive film, then centrifuge at 6,000 × *g* for 10 minutes to dry the membrane.

3.6. Discard the S-Block.

3.7. Place the extraction plate on the elution tubes, then add 100 µL of AE Buffer to each well.

3.8. Seal the extraction plate with adhesive film, then incubate at room temperature for 2 minutes.

3.9. Centrifuge at 6,000 × *g* for 3 minutes, then discard the extraction plate.

The purified DNA is in the elution tubes.

Store the purified DNA at 2–8°C for immediate use or below –16°C for long-term storage.

Purify DNA using the NucleoSpin™ 8 Tissue/NucleoSpin™ 96 Tissue kit (manual method)

Before first use of the kit

- Reconstitute the B5 Buffer—Add the required volume of 96–100% ethanol according to the recommendations of the supplier.
- Reconstitute the PK—Add the required volume of PB Buffer according to the recommendations of the supplier.

Perform the purification procedure

1 Lyse, then homogenize the samples

1.1. Combine the following components in a lysis plate (MN Round-Well Block) or lysis strip, then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock-purified sample	Volume per positive control
Prepared sample	200 µL of supernatant	—	—
PBS (1X), pH 7.4	—	200 µL	—
4d – EPC Cox b. FE	—	—	200 µL
5 – IPC Cox b. FE	10 µL	10 µL	10 µL
BQ1 Buffer	200 µL	200 µL	200 µL
Proteinase K	25 µL	25 µL	25 µL

1.2. Pipet up and down 5 times to mix, then seal the plate with adhesive film.

1.3. Incubate at 70°C for 30 minutes or at 56°C for 16–18 hours.

1	Lyse, then homogenize the samples *(continued)*	**1.4.** Allow the samples to cool, then centrifuge the samples briefly to bring down condensation. **1.5.** Add 200 µL of 96–100% ethanol to each sample, then mix by pipetting up and down 5 times.
2	Bind the DNA to the column	**2.1.** Place a NucleoSpin™ Tissue Binding Plate (extraction plate) or NucleoSpin™ Tissue Binding Strip (extraction strip) on a new MN Square-Well Block, then transfer each lysate to the appropriate wells of the extraction plate/strip. **2.2.** Seal the extraction plate/strip with adhesive film, then centrifuge the assembly at 6,000 × *g* for 2 minutes. **2.3.** Discard the MN Square-Well Block, then place the extraction plate/strip on a new MN Square-Well Block.
3	Wash, then elute the DNA	**3.1.** Add 600 µL of BW Buffer to each well, seal with adhesive film, then centrifuge at 6,000 × *g* for 2 minutes. **3.2.** Discard the MN Square-Well Block, then place the extraction plate/strip on a new MN Square-Well Block. **3.3.** Add 900 µL of B5 Buffer to each well, seal with adhesive film, then centrifuge at 6,000 × *g* for 2 minutes. **3.4.** Discard the MN Square-Well Block, then place the extraction plate/strip on a new MN Square-Well Block. **3.5.** Centrifuge at 6,000 × *g* for 10 minutes to dry the membrane. **3.6.** Discard the MN Square-Well Block. **3.7.** Place the extraction plate/strip on an elution plate or strip, then add 100 µL of BE Buffer to each well. **3.8.** Seal the extraction plate/strip with adhesive film, then incubate at room temperature for 3 minutes. **3.9.** Centrifuge at 6,000 × *g* for 3 minutes, then discard the extraction plate/strip. The purified DNA is in the elution plate/strip. Store the purified DNA at 2–8°C for immediate use or below –16°C for long-term storage.

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

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

Follow this procedure for purification with the MagMAX™ CORE Nucleic Acid Purification Kit using the KingFisher™ Duo Prime or KingFisher™ mL instrument.

Required materials not supplied

Table 8 Materials required for processing on the KingFisher™ Duo Prime and KingFisher™ mL instruments

Item	Source[1]
Consumables for the KingFisher™ Duo Prime instrument	
KingFisher™ Duo Combi pack for Microtiter 96 Deepwell plate (tip combs, plates, and elution strips for 96 samples)	97003530
KingFisher™ Duo Elution Strip (40 pieces)[2]	97003520
KingFisher™ Duo 12-tip comb for Microtiter 96 Deepwell plate (50 pieces)[2]	97003500
KingFisher™ Flex Microtiter Deep-Well 96 plates[2]	95040460
Consumables for the KingFisher™ mL instrument	
KingFisher™ mL Tubes and tip combs (for 240 samples)	97002141
KingFisher™ mL Tip comb (800 pieces)	97002111
KingFisher™ mL Tube (20 x 45 pieces)	97002121

[1] 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.

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

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 protocol, 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 Solution 1, MagMAX™ CORE Wash Solution 2, and MagMAX™ CORE Elution Buffer to the indicated positions, according to your instrument.

Table 9 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 10 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. Select the appropriate script on the instrument (see “Download and install the script” on page 6).

4. Start the run, then load the prepared plates or tube strips into the instrument at the same time. The instrument does not prompt you to load items individually.

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.

Appendix B Documentation and support

Customer and technical support

Visit **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)

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) 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**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. MAN0019383

Revision	Date	Description
B.0	20 October 2022	• The amount of PK-treated sample was updated (see "Treat samples with the PK Solution" on page 7 and "Combine PK-treated samples with the Lysis/Binding/Bead/IPC Solution" on page 8). • The instructions to lyse, then homogenize for wipes or boot swabs was updated (see "Lyse, then homogenize the samples (wipes or boot swabs)" on page 10). • The protocols for the MagVet™ Universal Isolation Kit were removed from the document.
A.0	18 August 2020	New document translated from the French document (MAN0008769 Rev. B.0) with the following updates: • Added the MagMAX™ CORE Nucleic Acid Purification Kit protocol. • Made minor wording and formatting updates for consistency with related documents.

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