VetMAX™ Ruminant Abortion Screening Kit

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

Pub. No. MAN0019291 Rev. A.0

Species	Sample matrices	Test type
Ruminants	• Placental, vaginal, and cervical swabs • Organs (fetus, placenta)	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 DNA purification protocols for the eight main pathogens responsible for abortions in ruminants (*Coxiella burnetii*, *Chlamydophila* spp., *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter fetus*, *Leptospira* pathogens, *Anaplasma phagocytophila* and *Bovine herpesvirus 4*). The methods have been validated and optimized for downstream use with the Applied Biosystems™ VetMAX™ Ruminant Abortion Screening Kit (Cat. No. SARP).

- 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.

Sample selection

Sample type	Type of analysis	Quantity required and sampling equipment
Placental, vaginal, and cervical swabs	Individual	Sterile dry swab
Organ (fetus, placenta)	Individual	20–30 mg of tissue

Sample storage

Sample type	Storage
Placental, vaginal, and cervical swabs	After collection, maintain the samples at 2°C to 8°C until use (up to 48 hours). After elution of the swab, use the eluate directly to perform PCR. After use or after 48 hours, store samples below –16°C for up to 1 year, or below –70°C for long-term storage.
Organ (fetus, placenta)	After sampling, store as indicated: • Store samples at 2°C to 8°C if the analysis is to be performed within 24 hours of sampling. • Store samples below –16°C if the analysis is to be performed more than 24 hours after sampling. After use or after 24 hours, 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.

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
Consumables	
Aerosol-resistant, nuclease-free pipette tips	MLS
1.5-mL and 2.0-mL DNase/RNase-free microtubes	MLS
Reagents	
Nuclease-free water	AM12450
PBS (1X), pH 7.4	MLS

Table 2 Additional materials required for purification from organ samples

Item	Source
Equipment	
(Optional) Tissue homogenizer for bead-beating, one of the following, or equivalent: • Fisher Scientific™ Bead Mill 24 Homogenizer • Precellys™ 24 Homogenizer (Bertin) • FastPrep-24™ Instrument (MP Biomedical 116004500) • Mixer Mill 400 (Verder 207450001)	 • Fisher Scientific 15-340-163 • Bertin EQ03119.200.RD000.0 • Fisher Scientific MP116004500 • Fisher Scientific 08 418 241
Precision scale	MLS
PYREX™ Solid Glass Beads for Distillation Columns (3 mm), or equivalent 3-mm glass beads	Fisher Scientific™ 11-312-10A
Scalpels and metallic forceps (sterile)	MLS
Consumables	
Petri dish (sterile)	MLS

Additional materials required for automated nucleic acid purification

Table 3 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
Consumables	
Adhesive PCR Plate Foils, or equivalent	AB0626
Consumables for the KingFisher™ Flex and MagMAX™ Express-96 instruments: • KingFisher™ Deepwell 96 Plate • KingFisher™ 96 KF microplates • KingFisher™ 96 tip comb for DW magnets	 • 95040450 • 97002540 • 97002534
Consumables for the KingFisher™ Duo Prime and KingFisher™ mL instruments	See Table 8 on page 13.
Kits and reagents	
MagMAX™ CORE Nucleic Acid Purification Kit	A32700 or A32702
PBS, pH 7.4 (10X), RNase-free	AM9624

Table 4 Materials required for the MagVet™ Universal Isolation Kit

Item	Source
Instrument, one of the following:	
KingFisher™ Flex Purification System	Contact your local sales office.
MagMAX™ Express-96 Magnetic Particle Processor	
KingFisher™ mL Purification System	

Item	Source
Equipment	
Heat block at 70°C	MLS
Reagent reservoir	MLS
Kits and reagents	
MagVet™ Universal Isolation Kit	MV384
MBL2 Buffer	12143
Proteinase K (PK)	• Qiagen 19131 • Macherey Nagel 740396
Ethanol, 80%	MLS

Additional materials required for manual nucleic acid purification

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

Workflow

Automated and manual nucleic acid purification workflow

Prepare samples for purification

Prepare samples according to your purification method, then proceed to the purification protocol that is appropriate for your laboratory.

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

OR

Purify nucleic acid using the MagVet™ Universal Isolation Kit (automated method)

OR

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

OR

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

Procedural guidelines

Prepare at least one 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. Process the mock-purified sample concurrently with the test samples, using the same nucleic acid purification protocol.

Prepare samples for purification

1. Prepare samples as described.

Sample type	Purification method	Action
Placental, vaginal, or cervical swab	Automated or manual	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. Press the swab against the wall of the tube to squeeze out the liquid, then discard the swab. 5. Proceed with 200 µL of eluate.
Organ (fetus, placenta)[1]	Automated (with disruption)	**Bead-beating method:** Prepare organ samples using a tissue homogenizer. 1. Add the following components to a 2-mL tube: • 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. • Fisher Scientific™ Bead Mill 24 Homogenizer—6 m/s for 45 seconds • Mixer Mill 400—30 Hz for 2 minutes 3. Centrifuge at 1,000 × *g* for 2 minutes. 4. Proceed with 200 µL of supernatant.
		Non-bead-beating method: Prepare organ samples using a vortex mixer. 1. Finely mince the organ piece in a sterile petri dish, using sterile forceps and a scalpel. 2. Add the following components to a 2-mL tube: • Tissue (finely minced)—20 to 30 mg • PBS (1X), pH 7.4—1 mL • PYREX™ Solid Glass Beads for Distillation Columns (3 mm)—2 beads 3. Vortex vigorously. 4. Centrifuge at 1,000 × *g* for 2 minutes. 5. Proceed with 200 µL of supernatant.
	Manual (without disruption)	1. Finely mince the organ piece in a sterile petri dish, using sterile forceps and a scalpel. 2. Proceed with 20 to 30 mg of minced tissue.

[1] Select the preparation method that is appropriate for your laboratory.

2. Proceed to DNA purification with the appropriate volume of prepared sample.

- "Purify nucleic acid using the MagMAX™ CORE Nucleic Acid Purification Kit (automated method)" on page 5
- "Purify nucleic acid using the MagVet™ Universal Isolation Kit (automated method)" on page 9
- "Purify DNA using the QIAamp™ DNA Mini Kit (manual method)" on page 10
- "Purify DNA using the NucleoSpin™ Tissue kit (manual method)" on page 11

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

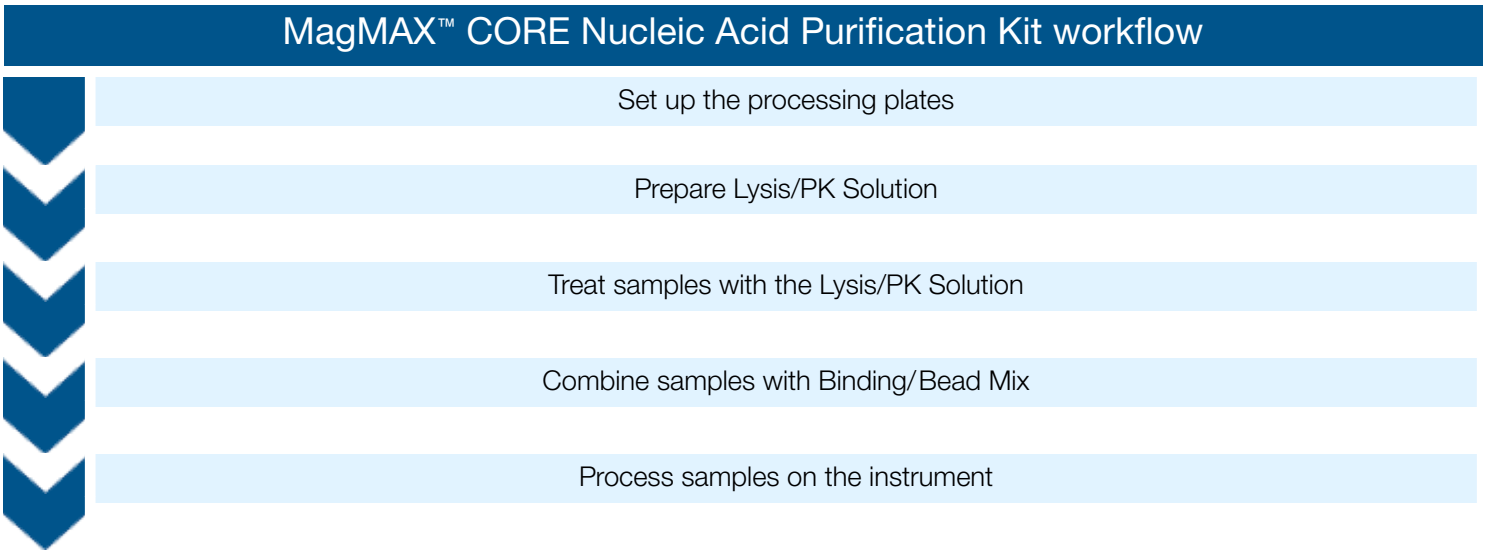
Follow this procedure if you are using these instruments:

- 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

Workflow



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.

Before first use of the kit

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

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

Download 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

a. 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".

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

2 Prepare Lysis/PK Solution

Prepare Lysis/PK Solution at the time of use. Do not mix in advance.

a. Combine the following components (in the order indicated) for the required number of samples plus 10% overage.

1. Combine the MagMAX™ CORE Lysis Solution with PBS (1X), pH 7.4.

Component	Volume per sample
MagMAX™ CORE Lysis Solution	200 µL
PBS (1X), pH 7.4	200 µL
Total diluted Lysis Solution	**400 µL**

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

3. Add MagMAX™ CORE Proteinase K to the diluted Lysis Solution.

Note: PK Buffer is not required for this protocol.

Component	Volume per sample
Diluted Lysis Solution	400 µL
MagMAX™ CORE Proteinase K	10 µL
Total Lysis/PK Solution	**410 µL**

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

3 Treat samples with the Lysis/PK Solution

Perform this procedure in single tubes to avoid contamination. Do not use plates.

a. Combine the following components in the order indicated.

Component	Volume per sample	Volume per mock-purified sample
Prepared sample	200 µL	—
PBS (1X), pH 7.4	—	200 µL
Lysis/PK Solution	410 µL	410 µL

b. Vortex briefly to mix the sample with the Lysis/PK Solution.

c. Incubate the sample according to the sample type.

For...	Do this...
Placental, vaginal, or cervical swab eluate	Incubate for 30 minutes at 55°C.
Organ supernatant	Incubate for 30 minutes to 2 hours at 55°C.

d. Centrifuge briefly to collect contents at the bottom of the tube.

4 Combine samples with Binding/Bead Mix

a. Transfer the entire volume of each sample lysate (up to 610 µL) to the appropriate wells in the sample plate or tube strip.

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

c. Prepare Binding/Bead Mix—Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Binding Solution	400 µL
MagMAX™ CORE Magnetic Beads	20 µL
Total Binding/Bead Mix	**420 µL**

d. Mix the Binding/Bead Mix by inversion until the solution is homogeneous, then add 420 µL of the Binding/Bead Mix to each sample.

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

5 Process samples on the instrument

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

b. 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.

Purify nucleic acid using the MagVet™ Universal Isolation Kit (automated method)

The following protocol can be used with the KingFisher™ Flex, KingFisher™ mL, and MagMAX™ Express-96 instruments.

Before first use of the kit

Note: PK and MBL2 Buffer must be ordered separately from the kit.

- Prepare the NM1 Lysis Buffer—Transfer 100 mL of N1 Buffer to the bottle of M1 Buffer (25 mL), then vortex to mix thoroughly.
  Store the NM1 Lysis Buffer at room temperature for up to 1 year.
- Reconstitute the PK—Follow the recommendations of the supplier.

Before each use of the kit

Prepare MBL2+Beads Mix—Combine the following components for the required number of samples plus 5–10% overage, then vortex to mix thoroughly.

Component	Volume per sample
MBL2 Buffer	500 µL
NM_LSI_Beads	20 µL

Discard the MBL2+Beads Mix after use.

Perform the purification procedure

1 Treat the lysate with PK

a. Combine the following components in the order indicated, then homogenize the sample.

Component	Volume per test sample	Volume per mock-purified sample
Prepared sample	200 µL	—
PBS (1X), pH 7.4	—	200 µL
Proteinase K	• Qiagen: 20 µL • Macherey Nagel: 25 µL	• Qiagen: 20 µL • Macherey Nagel: 25 µL
NM1 Lysis Buffer	280 µL	280 µL

b. Incubate at 70°C for 30 minutes.

2 Set up the processing plates or tube strips

Set up the processing plates or tube strips outside the instrument as described in the following table.

Position[1]	Plate type[2]	Reagent	Volume per well
2	Deep Well	NM3 Wash Buffer	600 µL
3	Deep Well	NM4 Wash Buffer	600 µL
4	Deep Well	80% ethanol	600 µL
5	Standard	NM6 Elution Buffer	200 µL
6	Deep Well	Place a tip comb in the plate or tube strip.	

[1] Position on the instrument.
[2] Does not apply if using tube strips.

3 Process samples on the instrument

a. When the 70°C incubation is complete, centrifuge the samples briefly to bring down condensation.

b. Transfer the entire volume of each sample lysate to the appropriate wells in the sample plate or tube strip.

c. Vortex the MBL2+Beads Mix thoroughly to ensure that the beads are fully resuspended.

d. Add 520 µL of MBL2+Beads Mix to each sample and control.

e. Select the appropriate script on the instrument.

- KingFisher™ Flex/MagMAX™ Express-96: **NM_LSI_RRC96**
- KingFisher™ mL: **NM_LSI_15prep**

f. Start the run, then load the prepared plates in the appropriate positions when prompted by the instrument.

Load the sample plate or tube strip at position 1 on the instrument.

Note: If you are using the KingFisher™ mL instrument, load the tip comb and all of the tube strips at the same time. The instrument does not prompt you to load items individually.

g. At the end of the run, when prompted by the instrument, remove the plate or tubes containing the purified nucleic acid.

Instrument	Procedure
• KingFisher™ Flex • MagMAX™ Express-96	Remove the plate at position 5, then cover with an adhesive film.
KingFisher™ mL	Remove the tube strip at position 5, then transfer the purified nucleic acid to new microcentrifuge tubes.

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

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

a. 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
Prepared sample	• 200 µL of swab eluate • 20–30 mg of minced tissue	—
PBS (1X), pH 7.4	—	200 µL
ATL Buffer	180 µL	180 µL
Proteinase K	20 µL	20 µL

b. Vortex for 15 seconds.

c. Incubate at 70℃ for 30 minutes or at 56℃ for 16–18 hours.

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

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

1	Lyse, then homogenize the samples *(continued)*	**f.** Incubate at 70℃ for 10 minutes. **g.** Allow the tubes to cool, then centrifuge briefly. **h.** Add 200 µL of 96–100% ethanol to each sample, vortex for 15 seconds, then briefly centrifuge to collect the contents.
2	Bind the DNA to the column	**a.** Insert a QIAamp™ DNA Mini Kit column into a collection tube, then transfer the entire sample volume to the column. **b.** Cap the column, then centrifuge the assembly at 15,000 × *g* for 1 minute. **c.** Discard the collection tube, then place the column on a new collection tube.
3	Wash, then elute the DNA	**a.** Add 500 µL of AW1 Buffer to each column, cap the column, then centrifuge at 15,000 × *g* for 1 minute. **b.** Discard the collection tube, then place the column on a new collection tube. **c.** Add 500 µL of AW2 Buffer to each column, cap the column, then centrifuge at 15,000 × *g* for 1 minute **d.** Discard the collection tube, then place the column on a new collection tube. **e.** Centrifuge at 15,000 × *g* for 3 minutes to dry the membrane. **f.** Discard the collection tube. **g.** Place the column on a new 1.5-mL microtube, then add 200 µL of AE Buffer. **h.** Cap the column, then incubate at room temperature for 1 minute. **i.** 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

a. 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
Prepared sample	• 200 µL of swab eluate • 20–30 mg of minced tissue	—
PBS (1X), pH 7.4	—	200 µL
T1 Buffer	180 µL	180 µL
Proteinase K	25 µL	25 µL

b. Vortex for 1 minute.

1	Lyse, then homogenize the samples *(continued)*	**c.** Incubate at 70℃ for 30 minutes or at 56℃ for 16–18 hours. **d.** Allow the tubes to cool, then centrifuge the samples briefly to bring down condensation. **e.** Add 200 µL of B3 Buffer, then vortex for 15 seconds. **f.** Incubate at 70℃ for 10 minutes. **g.** Allow the tubes to cool, then centrifuge briefly. **h.** Add 200 µL of 96–100% ethanol to each sample, vortex for 15 seconds, then briefly centrifuge to collect the contents.
2	Bind the DNA to the column	**a.** Insert a NucleoSpin™ Tissue kit column into a collection tube, then transfer the entire sample volume to the column. **b.** Cap the column, then centrifuge the assembly at 11,000 × *g* for 1 minute. **c.** Discard the collection tube, then place the column on a new collection tube.
3	Wash, then elute the DNA	**a.** Add 500 µL of BW Buffer to each column, cap the column, then centrifuge at 11,000 × *g* for 1 minute. **b.** Discard the collection tube, then place the column on a new collection tube. **c.** Add 600 µL of B5 Buffer to each column, cap the column, then centrifuge at 11,000 × *g* for 1 minute **d.** Discard the collection tube, then place the column on a new collection tube. **e.** Centrifuge at 11,000 × *g* for 3 minutes to dry the membrane. **f.** Discard the collection tube. **g.** Place the column on a new 1.5-mL microtube, then add 200 µL of BE Buffer. **h.** Cap the column, then incubate at room temperature for 1 minute. **i.** 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.

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 Deepwell 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 Solutions 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

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

Revision history: Pub. No. MAN0019291

Revision	Date	Description
A.0	29 June 2020	New document translated from the French document (MAN0008873 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.