VetMAX™ S. enterica spp. Kit

Nucleic acid purification protocols validated (according to PCR methods NF U47-600) and optimized for use with the kit (Cat. No. SALMSPP, SALMSPP50)

Pub. No. MAN0019475 Rev. A.0

Species	Sample matrices	Test type
Poultry	• Wipes and boot swabs • Feces, slurry, and manure • Bedding, bottom cage compartments, and soil • Organs and embryos	Individual
Bovine	• Organs (placental biopsy, fetal spleen) • Placental and cervical swabs • Liquids (fetal stomach contents, vaginal mucus) • Feces • Milk	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.

For Laboratory Use.

Purpose of this guide

This guide describes *Salmonella enterica* spp. bacterial DNA purification protocols that have been validated (according to PCR methods NF U47-600) and optimized for downstream use with the Applied Biosystems™ VetMAX™ S. enterica spp. Kit (Cat. No. SALMSPP, SALMSPP50).

- 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
Abortion application		
Organs	Individual	20 mg
Placental and cervical swabs	Individual	200 µL of eluate collected from a 20–25 cm cotton swab
Liquids	Individual	• 200 µL of fetal stomach contents • 200 µL of vaginal mucus
Poultry application		
Wipes	Individual	20–60 g
Boot swabs	Individual	20–60 g
Dust	Individual	20–40 g
Feces, slurry, and manure	Individual	50–100 g
Bottom cage compartments, crate, and bedding	Individual	Five, 30 cm × 30 cm units
Embryos	Combination	30 embryos maximum
Organs	Combination	10 similar organs maximum
Livestock-monitoring application		
Feces	Individual	50–100 g
Milk	Individual	200 µL minimum

Sample storage

Sample type	Storage
Organs (placental biopsy, fetal spleen)	After collection, use the fresh samples immediately or store at 2°C to 8°C until use (up to 8 days). After use or after 8 days, store samples below –16°C for up to 1 month, or below –70°C for long-term storage.
Placental 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 nucleic acid purification. After use or after 48 hours, store samples below –16°C for up to 1 year, or below –70°C for long-term storage.
Liquids (fetal stomach contents, vaginal mucus)	After collection, maintain the samples at 2°C to 8°C until use (up to 8 days). After use or after 8 days, store samples below –16°C for up to 1 year, or below –70°C for long-term storage.
• Wipes • Boot swabs • Dust • Slurry and manure • Bottom cage compartment, crate, and bedding	After collection, use the fresh samples immediately or store at 2°C to 8°C until use (up to 8 days). After use or after 8 days, store samples below –16°C for up to 1 month, or below –70°C for long-term storage.

Sample type	Storage
Embryos and organs	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.
Feces	After collection, maintain the samples at 2°C to 8°C until use (up to 8 days). After use or after 8 days, store samples below –16°C for up to 1 month, or below –70°C for long-term storage.
Milk	After collection, immediately add preservatives, then maintain the samples at 2°C to 8°C until use (up to 8 days). After use or after 8 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.

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	
5 – IPC Salmo	From the VetMAX™ S. enterica spp. Kit (Cat. No. SALMSPP, SALMSPP50)
Nuclease-free water	AM9932
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

Table 3 Additional materials required for sample enrichment (for poultry and livestock-monitoring applications)

Item	Source
Equipment	
Stomacher™ Model 3500 Jumbo Lab Blender, or equivalent peristaltic laboratory blender	Fisher Scientific 14-285-32
Stomacher™ 400 homogenizer bag rack, or equivalent	Fisher Scientific 14-285-3
Stomacher™ 400 homogenizer bag clips, or equivalent	Fisher Scientific 13-874-850
Consumables	
Stomacher™ 400 homogenizer bags, or equivalent	Fisher Scientific 14-285-24
Reagents	
Buffered Peptone Water (BPW)	CM0509T

Additional materials required for automated nucleic acid purification

Table 4 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 9 on page 18.
Kits and reagents	
MagMAX™ CORE Nucleic Acid Purification Kit	A32700 or A32702
PBS, pH 7.4 (10X), RNase-free	AM9624

Table 5 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	
Equipment	
Heat block at 70°C	MLS
Reagent reservoir	MLS

Item	Source
Kits and reagents	
MagVet™ Universal Isolation Kit	MV384
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 • NucleoSpin™ 96 Tissue kit • NucleoSpin™ 8 Tissue kit	 • Qiagen 51304 • Macherey Nagel 740952 • Macherey Nagel 740741.4 • Macherey Nagel 740740
Ethanol, 96–100%	MLS

Recommended DNA purification protocols

Purification protocol	Application		
	Abortion	Poultry	Livestock-monitoring
MagMAX™ CORE Nucleic Acid Purification Kit (page 6)	✓	—	—
MagVet™ Universal Isolation Kit (page 12)	✓	✓	✓
QIAamp™ DNA Mini Kit (page 13)	✓	✓	✓
NucleoSpin™ Tissue kit (page 15)	✓	✓	✓
NucleoSpin™ 8 Tissue/NucleoSpin™ 96 Tissue kit (page 16)	✓	✓	✓

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.

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

This procedure is designed for rapid purification of bacterial DNA from the following sample types for the abortion application.

- Organs (placental biopsy, fetal spleen)
- Placental and cervical swabs
- Liquids (fetal stomach contents, vaginal mucus)

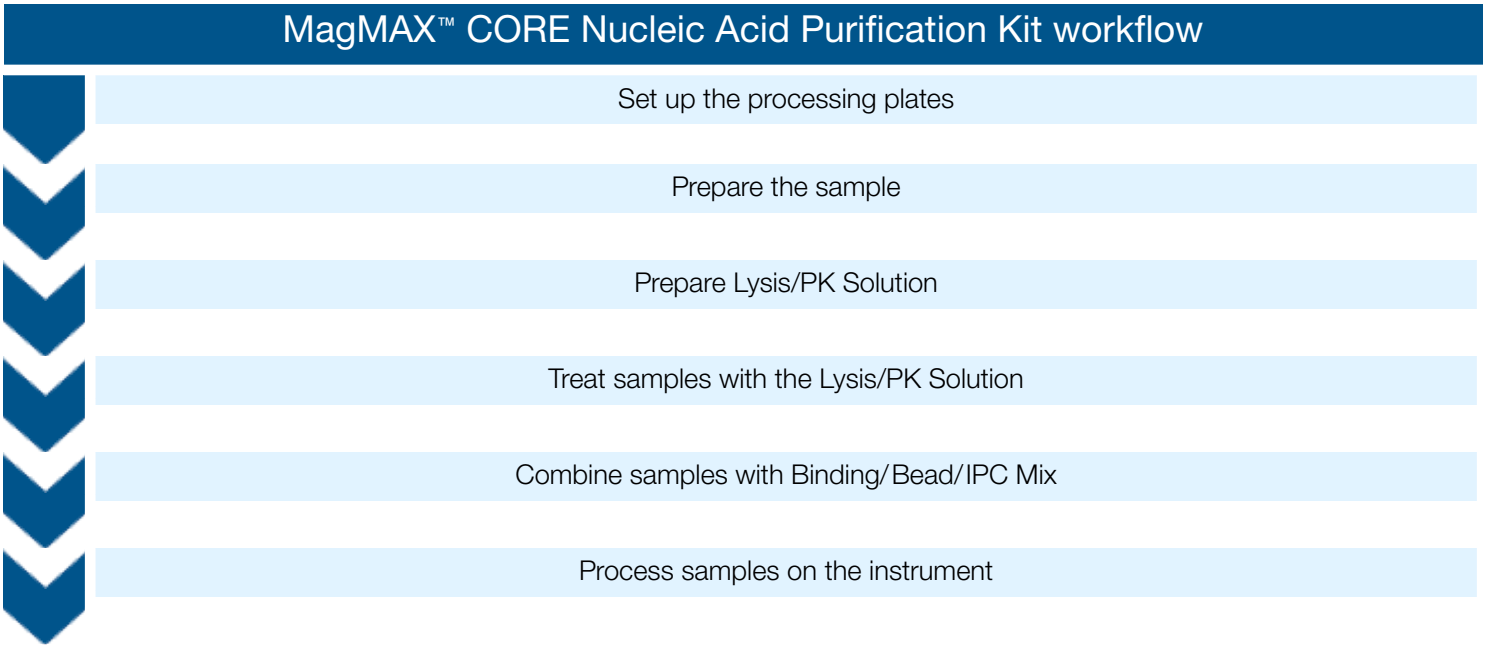
Follow this procedure if you are using these instruments:

- 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 6 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 7 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 8 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 the sample

Prepare samples as described.

Sample type	Action
Organ (placental biopsy, fetal spleen)[1]	**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.
Placental or cervical swab	1. Break off the tip of the swab, then add the swab to a 2-mL tube. 2. Add 1 mL of PBS (1X), pH 7.4, to each sample. 3. Vortex vigorously. 4. Proceed with 200 µL of eluate.
Liquid (fetal stomach contents, vaginal mucus)	Proceed with 200 µL of liquid.

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

3 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 Prepare Lysis/PK Solution *(continued)*

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.

4 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 or cervical swab eluate • Liquid (fetal stomach contents, vaginal mucus)	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.

5 Combine samples with Binding/Bead/IPC 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/IPC 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
5 – IPC Salmo	5 µL
Total Binding/Bead/IPC Mix	**425 µL**

d. Mix the Binding/Bead/IPC Mix by inversion until the solution is homogeneous, then add 425 µL of the Binding/Bead/IPC Mix to each sample.

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

6	Process samples on the instrument	**a.** Select the appropriate script on the instrument (see "Download and install the script" on page 7). **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.

Prepare samples for purification with other kits

Follow the appropriate sample preparation procedure according to your application.

- **Abortion application—**See "Prepare samples: Abortion application" on page 10.
- **Poultry application—**See "Prepare samples for enrichment: Poultry application" on page 11.
- **Livestock-monitoring application—**See "Prepare samples for enrichment: Livestock-monitoring application" on page 11.

Note: A sample-enrichment phase is required for poultry and livestock-monitoring applications.

Prepare samples: Abortion application

1. Prepare samples as described.

Sample type	Action
Organ (placental biopsy, fetal spleen)[1]	**Bead-beating method:** Prepare organ samples using a tissue homogenizer. 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—20 mg • Nuclease-free water—1 mL • PYREX™ Solid Glass Beads for Distillation Columns (3 mm)—3 beads 3. Disrupt (bead-beat) the samples in a Mixer Mill 400 homogenizer (or equivalent) at 30 Hz for 2 × 5 minutes. 4. Centrifuge at 1,500 × *g* for 2 minutes. 5. Proceed with 100 µL of supernatant.
	Non-bead-beating method: Prepare organ samples without disruption. 1. Finely mince the organ piece in a sterile petri dish, using sterile forceps and a scalpel. 2. Proceed with 20 mg of minced tissue.
Placental or cervical swab	1. Break off the tip of the swab, then add the swab to a 2-mL tube. 2. Add 1 mL of PBS (1X), pH 7.4, to each sample. 3. Vortex vigorously. 4. Proceed with 200 µL of eluate.
Liquid (fetal stomach contents, vaginal mucus)	Proceed with 200 µL of liquid.

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

2. Proceed to DNA purification with the appropriate volume of sample.
   - "Purify nucleic acid using the MagVet™ Universal Isolation Kit (automated method)" on page 12
   - "Purify DNA using the QIAamp™ DNA Mini Kit (manual method)" on page 13
   - "Purify DNA using the NucleoSpin™ Tissue kit (manual method)" on page 15
   - "Purify DNA using the NucleoSpin™ 8 Tissue/NucleoSpin™ 96 Tissue kit (manual method)" on page 16

Prepare samples for enrichment: Poultry application

1. Determine the approximate weight of each sample, then transfer to a sterile homogenizer bag.
2. Based on the sample weight, add the appropriate volume of buffered peptone water according to the following table.

Sample type	Volume per sample
Wipes (20–60 g)	100–600 mL[1]
Boot swabs (20–60 g)	100–150 mL[1]
Dust (20–40 g)	100–200 mL
Feces (50–100 g)	250–500 mL
Slurry or manure (50–100 g)	250–500 mL
Bottom cage compartment, crate, or bedding (five, 30 cm × 30 cm units)	1–2 L
Embryos (up to 30)	10–20% (m/m or v/v)
Organs (up to 10)	10–20% (m/m or v/v)

[1] Ensure that the sample is completely submerged in the buffered peptone water.

3. Proceed to enrich the samples (see "Enrich samples (poultry and livestock-monitoring applications)" on page 11).

Prepare samples for enrichment: Livestock-monitoring application

Prepare samples as described.

Sample type	Action
Feces[1]	**Clinical cases:** Follow this procedure to prepare fecal samples from symptomatic animals. 1. Add the following components to a 10-mL tube: • Feces—1 g of fresh or frozen sample • Nuclease-free water—4 mL 2. Vortex vigorously for 1 minute. 3. Allow the contents of the tube to settle for 5–10 minutes. 4. Transfer 1 mL of the supernatant to a 1.5-mL microtube. 5. Centrifuge at 10,000 × *g* for 2 minutes. 6. Proceed directly to DNA purification with 200 µL of supernatant. An enrichment phase is not required.
	Routine monitoring: Follow this procedure to prepare fecal samples for routine monitoring of livestock. 1. Add 50–100 g of feces to a sterile homogenizer bag. 2. Proceed to enrich the samples (see "Enrich samples (poultry and livestock-monitoring applications)" on page 11).
Milk	1. Add 200 µL of milk to a sterile homogenizer bag. 2. Proceed to enrich the samples (see "Enrich samples (poultry and livestock-monitoring applications)" on page 11).

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

Enrich samples (poultry and livestock-monitoring applications)

1. Pre-warm buffered peptone water to 37°C before inoculating the samples.
2. To each sample, add the appropriate volume of pre-warmed buffered peptone water to achieve a 10% (m/m or v/v) dilution of the sample in the enrichment medium.
3. Homogenize the sample for a minimum of 1 minute in a peristaltic laboratory blender.
4. Incubate the sample for 18±2 hours at 37°C.
5. Homogenize the sample for a minimum of 1 minute in a peristaltic laboratory blender.

6. Centrifuge an aliquot of each sample according to your purification method.
   - For automated purification—Transfer 5 mL of the inoculum to a 10-mL tube, then centrifuge at 5,000 × *g* for 10 minutes.
   - For manual purification—Transfer 1.5 mL of the inoculum to a 2-mL tube, then centrifuge at 4,500 × *g* for 10 minutes.
7. Remove the supernatant, then resuspend the pellet in 200 µL of PBS (1X), pH 7.4.

   Note: Do not remove fat if it is present in the sample. Bacteria is often in the fatty tissue.
8. Proceed to DNA purification with 200 µL of the enriched pellet in 1X PBS.

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 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 NM2+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
NM2 Binding Solution	600 µL
NM_LSI_Beads	20 µL

Discard the NM2+Beads Mix after use.

Perform the purification procedure

1 Treat the lysate with PK

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

For...	Use...
Abortion samples	• 100 µL of organ supernatant • 20 mg of minced tissue • 200 µL of liquid or swab eluate
Poultry samples	200 µL of enriched pellet in 1X PBS
Livestock-monitoring samples	• 200 µL of enriched pellet in 1X PBS • 200 µL of fecal supernatant
Mock-purified sample	200 µL of PBS (1X), pH 7.4

b. Add the following components to each sample in the order indicated, then homogenize the sample.

Component	Volume per sample
5 – IPC Salmo	5 µL
Proteinase K	20 µL (Qiagen) or 25 µL (Macherey Nagel)
NM1 Lysis Buffer	180 µL

c. 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 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 NM2+Beads Mix thoroughly to ensure that the beads are fully resuspended.

d. Add 620 µL of the NM2+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. Transfer the appropriate volume of each prepared sample to a new tube.

For...	Use...
Abortion samples	• 100 µL of organ supernatant • 20 mg of minced tissue • 200 µL of liquid or swab eluate
Poultry samples	200 µL of enriched pellet in 1X PBS
Livestock-monitoring samples	• 200 µL of enriched pellet in 1X PBS • 200 µL of fecal supernatant
Mock-purified sample	200 µL of PBS (1X), pH 7.4

b. Add the following components to each sample in the order indicated, then immediately proceed to the next step.

Component	Volume per sample
5 – IPC Salmo	5 µL
Proteinase K	20 µL
ATL Buffer	180 µL

c. Vortex for 15 seconds.

d. Incubate at 70°C for 30 minutes.

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

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

g. Incubate at 70°C for 10 minutes.

h. Allow the tubes to cool, then centrifuge briefly.

i. 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.

3 Wash, then elute the DNA *(continued)*

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. Transfer the appropriate volume of each prepared sample to a new tube.

For...	Use...
Abortion samples	• 100 µL of organ supernatant • 20 mg of minced tissue • 200 µL of liquid or swab eluate
Poultry samples	200 µL of enriched pellet in 1X PBS
Livestock-monitoring samples	• 200 µL of enriched pellet in 1X PBS • 200 µL of fecal supernatant
Mock-purified sample	200 µL of PBS (1X), pH 7.4

b. Add the following components to each sample in the order indicated, then immediately proceed to the next step.

Component	Volume per sample
5 – IPC Salmo	5 µL
Proteinase K	25 µL
T1 Buffer	180 µL

c. Vortex for 15 seconds.

d. Incubate at 70°C for 30 minutes.

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

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

g. Incubate at 70°C for 10 minutes.

h. Allow the tubes to cool, then centrifuge briefly.

i. 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 500 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 11,000 × *g* for 2 minutes, 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™ 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

a. Transfer the appropriate volume of each prepared sample to a lysis plate (MN Round-Well Block) or lysis strip (rack of tube strips).

For...	Use...
Abortion samples	• 100 µL of organ supernatant • 20 mg of minced tissue • 200 µL of liquid or swab eluate
Poultry samples	200 µL of enriched pellet in 1X PBS
Livestock-monitoring samples	• 200 µL of enriched pellet in 1X PBS • 200 µL of fecal supernatant
Mock-purified sample	200 µL of PBS (1X), pH 7.4

1 Lyse, then homogenize the samples *(continued)*

b. Add the following components to each sample in the order indicated, then immediately proceed to the next step.

Component	Volume per sample
5 – IPC Salmo	5 µL
Proteinase K	25 µL
BQ1 Buffer	180 µL

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

d. Incubate at 70°C for 30 minutes.

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

f. 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

a. 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.

b. Seal the extraction plate/strip with adhesive film, then centrifuge the assembly at 6,000 × *g* for 5 minutes.

c. 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

Preheat an aliquot of BE Buffer to 70°C.

a. Add 500 µL of BW Buffer to each well, seal with adhesive film, then centrifuge at 6,000 × *g* for 3 minutes.

b. Discard the MN Square-Well Block, then place the extraction plate/strip on a new MN Square-Well Block.

c. Add 700 µL of B5 Buffer to each well, seal with adhesive film, then centrifuge at 6,000 × *g* for 2 minutes.

d. Discard the MN Square-Well Block.

e. Place the extraction plate/strip on an elution plate or strip, then add 200 µL of BE Buffer (preheated to 70°C) to each well.

f. Seal the extraction plate/strip with adhesive film, then incubate at room temperature for 1 minute.

g. Centrifuge at 6,000 × *g* for 2 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 9 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 10 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 11 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 7).

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

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Revision	Date	Description
A.0	4 September 2020	New document translated from the French document (MAN0008869 Rev. B.0) with the following updates: • Added the MagMAX™ CORE Nucleic Acid Purification Kit protocol. • Removed VersaTREK™ liquid cultures from the sample storage section. • Made minor wording and formatting updates for consistency with related documents.