VetMAX™ EHDV Kit

Nucleic acid purification protocols optimized for use with the kit (Cat. No. EHDV50)

Catalog Number EHDV50

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Species	Sample matrices	Test type
• Bovine • Small ruminants (sheep, goats)	• EDTA-treated whole blood • Spleen or organs from aborted animals (spleen, liver, heart)	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 epizootic hemorrhagic disease virus (EHDV) viral RNA purification protocols that have been validated and optimized for downstream use with the Applied Biosystems™ VetMAX™ EHDV Kit (Cat. No. EHDV50).

- 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 and sampling equipment
Whole blood	Individual	50–200 µL of whole blood collected in EDTA tubes[1]
Organ	Individual	1 g of spleen, liver or heart

[1] The volume required depends on the purification protocol used.

Sample storage

Sample type	Storage
Whole blood	After collection, maintain the samples at 2°C to 8°C until use (up to 4 days). After use or after 4 days, store samples below –16°C for up to 1 year, or below –70°C for long-term storage. **Note:** In France, store blood samples according to Appendix A, "Blood storage guidelines applicable to France".
Organ	After sampling, store samples at 2°C to 8°C if the analysis is to be performed within 24 hours of 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.

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

Table 2 Additional materials required for purification from tissue samples

Item	Source
Equipment	
Tissue homogenizer for bead-beating, one of the following, or equivalent: • Fisherbrand™ Bead Mill 24 Homogenizer • Precellys™ 24 Homogenizer (Bertin) • FastPrep-24™ Instrument (MP Biomedical 116004500) • Mixer Mill MM 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	
Reagent reservoir	MLS
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 9 on page 19.
Kits and reagents	
MagMAX™ CORE Nucleic Acid Purification Kit	A32700 or A32702

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	
Equipment	
Reagent reservoir	MLS

Item	Source
Kits and reagents	
MagVet™ Universal Isolation Kit	MV384
Ethanol, 80%	MLS

Table 5 Materials required for the MagMAX™-96 Viral RNA Isolation Kit

Item	Source
Instrument	
MagMAX™ Express-96 Magnetic Particle Processor	Contact your local sales office.
Equipment	
Reagent reservoir	MLS
Kits and reagents	
MagMAX™-96 Viral RNA Isolation Kit	AM1836
Ethanol, 96–100%	MLS
Isopropanol, 100%	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™ Viral RNA Mini Kit • NucleoSpin™ RNA Virus kit • NucleoSpin™ 96 Virus kit • NucleoSpin™ 8 Virus kit	 • Qiagen 52904 • Macherey Nagel 740956 • Macherey Nagel 740691.4 • Macherey Nagel 740643
Ethanol, 96–100%	MLS

Recommended RNA purification kits

	Recommended purification kits
Whole blood	MagMAX™ CORE Nucleic Acid Purification Kit
	MagVet™ Universal Isolation Kit
	MagMAX™-96 Viral RNA Isolation Kit
	QIAamp™ Viral RNA Mini Kit
	NucleoSpin™ RNA Virus
	NucleoSpin™ 8 / 96 Virus
Organ samples	QIAamp™ Viral RNA Mini Kit
	NucleoSpin™ RNA Virus
	NucleoSpin™ 8 / 96 Virus

Procedural guidelines

Prepare at least one mock sample for use as a negative extraction control—use PBS, or nuclease-free water in place of the test sample, unless otherwise directed. Process the mock sample concurrently with the test samples, using the same nucleic acid purification protocol.

Prepare samples for purification (all methods)

1. Prepare samples as described.

Sample type	Action
Whole blood	Proceed with 50–200 µL of sample, depending on the purification protocol used.
Organ sample	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: • Organ sample—1 g • 1X PBS, pH 7.4—10 mL • PYREX™ Solid Glass Beads for Distillation Columns (3 mm)—2 beads 3. Disrupt (bead-beat) the samples. • Precellys™ 24 Homogenizer—6,000 rpm for 40 seconds • FastPrep-24™ Instrument—6 m/s for 45 seconds • Mixer Mill MM 400—30 Hz for 2 minutes 4. Centrifuge at 1,000 × *g* for 2 minutes at 4°C. 5. Proceed with 50–100 µL of supernatant, depending on the purification protocol used.

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

- "Purify nucleic acid using the MagMAX™ CORE Nucleic Acid Purification Kit" on page 5
- "Purify nucleic acid using the MagVet™ Universal Isolation Kit (automated method)" on page 12
- "Purify RNA using the MagMAX™-96 Viral RNA Isolation Kit (automated method)" on page 13
- "Purify RNA using the QIAamp™ Viral RNA Mini Kit (manual method)" on page 15
- "Purify RNA using the NucleoSpin™ RNA Virus kit (manual method)" on page 16
- "Purify RNA using the NucleoSpin™ 8 Virus/NucleoSpin™ 96 Virus kit (manual method)" on page 17

Purify nucleic acid using the MagMAX™ CORE Nucleic Acid Purification Kit

This protocol is intended for purification only of viral RNA from whole blood samples in EDTA tubes.

Follow this procedure if you are using these instruments:

- KingFisher™ Flex
- MagMAX™ Express-96

Follow Appendix B, "Purify nucleic acid using the KingFisher™ Duo Prime or KingFisher™ mL instrument" if you are using these instruments:

- KingFisher™ Duo Prime
- KingFisher™ mL

Recommended workflows for use with the MagMAX™ CORE Nucleic Acid Purification Kit

Three workflow options are available for the purification of whole blood samples using the MagMAX™ CORE Nucleic Acid Purification Kit:

- **Multisample Simple Workflow**—Recommended for processing a combination of sample types.
- **Simple Workflow**—Recommended for processing blood samples.
- **Whole Blood Workflow**—Recommended only for processing whole blood samples.

The Multisample Simple and Simple Workflows only differ in the order that the reagents are added to the samples.

Note: An express script is available for processing samples on the instrument (see "Download and install the script" on page 8).

Workflow: Multisample Simple

Multisample Simple Workflow

- **Set up the processing plates**
- **Prepare Lysis/Binding/Bead Mix**
- **Combine samples with PK, then add the Lysis/Binding/Bead Mix**
- **Process samples on the instrument**

Workflow: Simple

Simple Workflow

- **Set up the processing plates**
- **Prepare Bead/PK Mix**
- **Prepare Lysis/Binding Mix**
- **Combine samples with the Bead/PK Mix and Lysis/Binding Mix**
- **Process samples on the instrument**

Whole Blood Workflow

Set up the processing plates

Prepare PK/PBS Mix

Prepare Lysis/Binding/Bead Mix

Prepare the sample

Combine the sample with PK/PBS Mix, then add the Lysis/Binding/Bead Mix

Process samples on the instrument

Procedural guidelines

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

  Note: Do not use a plate shaker with the tube strips that are required by the KingFisher™ mL instrument.
- To prevent cross-contamination:
  - Cover the plate or tube strip during the incubation and shaking steps, to prevent spill-over.
  - Carefully pipet reagents and samples, to avoid splashing.
- To prevent nuclease contamination:
  - Wear laboratory gloves during the procedures. Gloves protect you from the reagents, and they protect the nucleic acid from nucleases that are present on skin.
  - Use nucleic acid-free pipette tips to handle the reagents, and avoid putting used tips into the reagent containers.
  - Decontaminate lab benches and pipettes before you begin.

Before first use of the kit

Determine the maximum plate shaker setting

If a plate shaker is used, use the following steps to determine the maximum setting.

1. Verify that the plate fits securely on your shaker.
2. Add 1 mL of water to each well of the plate, then cover with sealing foil.
3. Determine the maximum setting that you can use on your shaker without any of the water splashing onto the sealing foil.

Download and install the script

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

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

2. Locate and download the latest version of the appropriate file, then download the latest version of the `MagMAX_CORE` script for your instrument.

   Table 6 Recommended scripts

Instrument	Script name	
	Standard script	Express script[1]
KingFisher™ Flex	`MagMAX_CORE_Flex.bdz`	`MagMAX_CORE_Flex_Express.bdz`
KingFisher™ 96 MagMAX™ Express-96	`MagMAX_CORE_KF-96.bdz`	`MMC_KF96_Express.kf2`
KingFisher™ Duo Prime	`MagMAX_CORE_DUO.bdz`	`MagMAX_CORE_DUO_Express.bdz`
KingFisher™ mL	`MagMAX_CORE_mL_no_heat.bdz`	`MagMAX_CORE_mL_Express.bdz`

[1] The Express scripts do not have heating steps.

   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	
	Standard script	Express script
KingFisher™ Flex	`MagMAX_CORE_Flex_no_heat.bdz`	`MagMAX_CORE_Flex_Express.bdz`
KingFisher™ 96 MagMAX™ Express-96	`MagMAX_CORE_KF-96_no_heat.bdz`	`MMC_KF96_Express.kf2`
KingFisher™ Duo Prime	`MagMAX_CORE_DUO_no_heat.bdz`	`MagMAX_CORE_DUO_Express.bdz`
KingFisher™ mL	`MagMAX_CORE_mL_no_heat.bdz`	`MagMAX_CORE_mL_Express.bdz`

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

Set up the processing plates

1. 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 page 19.

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

3. Proceed to the workflow that is appropriate for your laboratory.
   - **Multisample Simple Workflow**—See "Multisample Simple Workflow: Prepare samples for processing" on page 9.
   - **Simple Workflow**—See "Simple Workflow: Prepare samples for processing" on page 10.
   - **Whole Blood Workflow**—See "Whole Blood Workflow: Prepare samples for processing" on page 11

Multisample Simple Workflow: Prepare samples for processing

1 Prepare Lysis/Binding/Bead Mix

1. Vortex the MagMAX™ CORE Magnetic Beads thoroughly to ensure that the beads are fully resuspended.
2. Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Lysis Solution	350 µL
MagMAX™ CORE Binding Solution	350 µL
MagMAX™ CORE Magnetic Beads	20 µL
Total Lysis/Binding/Bead Mix	**720 µL**

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

2 Combine samples with PK, then add the Lysis/Binding/Bead Mix

1. Add 10 µL of MagMAX™ CORE Proteinase K to the appropriate wells in the sample plate or tube strip.
2. Transfer 200 µL of each whole blood sample to a well or tube with MagMAX™ CORE Proteinase K.

2 Combine samples with PK, then add the Lysis/Binding/Bead Mix *(continued)*

3. Mix the sample with Proteinase K for 2 minutes at room temperature according to your mixing method.
   - **Using a plate shaker**—Shake vigorously for 2 minutes (see "Determine the maximum plate shaker setting" on page 8).
   - **By pipetting**—Pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL instrument, you must mix by pipetting.)
4. Invert the tube of Lysis/Binding/Bead Mix several times to resuspend the beads, then add 720 µL of Lysis/Binding/Bead Mix to each sample.
5. Immediately proceed to "Process samples on the instrument" on page 12.

   Note: If you are using the KingFisher™ Duo Prime or KingFisher™ mL instrument, proceed to "Set up the processing plates" on page 9.

Simple Workflow: Prepare samples for processing

1 **Prepare Bead/PK Mix**

Prepare new Bead/PK Mix for each processing run.

1. Vortex the MagMAX™ CORE Magnetic Beads thoroughly to ensure that the beads are fully resuspended.
2. Combine the following components for the required number of samples, plus 10% overage (recommended).

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

(Optional) Store the Bead/PK Mix at 4°C for up to 1 week.

2 **Prepare Lysis/Binding Mix**

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

Component	Volume per sample
MagMAX™ CORE Lysis Solution	350 µL
MagMAX™ CORE Binding Solution	350 µL
Total Lysis/Binding Mix	**700 µL**

2. Invert the tube or bottle at least 10 times to mix.

3 **Combine samples with the Bead/PK Mix and Lysis/Binding Mix**

1. Invert the tube of Bead/PK Mix several times to resuspend the beads, then add 30 µL of the Bead/PK Mix to the appropriate wells in the sample plate or tube strip.
2. Transfer 200 µL of each whole blood sample to a well or tube with Bead/PK Mix.
3. Mix the sample with the Bead/PK Mix for 2 minutes at room temperature according to your mixing method.
   - **Using a plate shaker**—Shake vigorously for 2 minutes (see "Determine the maximum plate shaker setting" on page 8).
   - **By pipetting**—Pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL instrument, you must mix by pipetting.)
4. Add 700 µL of the Lysis/Binding Mix to each sample-containing well or tube.

3 Combine samples with the Bead/ PK Mix and Lysis/Binding Mix *(continued)*

5. Immediately proceed to "Process samples on the instrument" on page 12.

   Note: If you are using the KingFisher™ Duo Prime or KingFisher™ mL instrument, proceed to "Set up the processing plates" on page 9.

Whole Blood Workflow: Prepare samples for processing

1 Prepare PK/PBS Mix

Prepare new PK/PBS Mix for each processing run.

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

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

2 Prepare Lysis/Binding/Bead Mix

1. Vortex the MagMAX™ CORE Magnetic Beads thoroughly to ensure that the beads are fully resuspended.
2. Combine the following components for the required number of samples, plus 10% overage (recommended).

Component	Volume per sample
MagMAX™ CORE Lysis Solution	350 µL
MagMAX™ CORE Binding Solution	350 µL
MagMAX™ CORE Magnetic Beads	20 µL
Total Lysis/Binding/Bead Mix	**720 µL**

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

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

3 Prepare the sample

Prepare samples and controls as described.

Sample type	Action
Whole blood	Proceed with 100 µL of sample.
NCS	—

4 Combine the sample with PK/PBS Mix, then add the Lysis/Binding/Bead Mix

1. Invert the tube of PK/PBS Mix several times to mix, then add 200 µL of the PK/PBS Mix to the required wells in the plate or tube strip.
2. Transfer 100 µL of each sample to a well with PK/PBS Mix.
3. Mix the sample with PK/PBS Mix at room temperature by vortexing, or pipetting up and down 3 times.

   (For downstream processing on the KingFisher™ mL instrument, you must mix by pipetting.)
4. Incubate at room temperature for 5 minutes.
5. Add 720 µL of Lysis/Binding/Bead Mix to each sample-containing well or tube strip.
6. Immediately proceed to "Process samples on the instrument" on page 12.

   Note: If you are using the KingFisher™ Duo Prime or KingFisher™ mL instrument, proceed to "Set up the processing plates" on page 9.

Process samples on the instrument

1. Select the appropriate script on the instrument (see "Download and install the script" on page 8).

   Note: For rapid processing of samples, select one of the following express scripts on the instrument.
   - KingFisher™ Flex: **MagMAX_CORE_Flex_Express.bdz**
   - KingFisher™ 96/MagMAX™ Express-96: **MMC_KF96_Express.kf2**

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.
- For samples processed with an express script, perform real-time RT-PCR using the express thermal-cycling method (see the *VetMAX™ EHDV Kit Instructions for Use* (Pub. No. MAN0008223)).

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

- Prepare the NM1 Lysis Buffer—Transfer 100 mL of N1 solution to the bottle of M1 solution (25 mL), then vortex to mix thoroughly.

  Store the NM1 Lysis Buffer at room temperature for up to 1 year.

Before each use of the kit

Prepare NM2+Beads solution—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 Buffer	600 µL
NM_LSI_Beads	20 µL

Discard the NM2+Beads solution after use.

Perform the purification procedure

1 Prepare the consumables

Prepare the following consumables for the purification.
- KingFisher™ Flex/MagMAX™ Express-96: Add the buffers to the plates on the bench.
- KingFisher™ mL: Remove the system's tube-strip tray and place the tray on the bench. Place the extraction strips on the tray, then add the buffers.

2 Lyse the samples

Add the following components to the appropriate wells in the sample plate or tube strip (plate 1 or position A of the strip are recommended).

Component	Volume per test sample	Volume per mock sample
Prepared sample	100 µL of whole blood or serum	—
NM1 Lysis solution	250 µL	250 µL

3 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	80 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.

4 Process samples on the instrument

1. Add 620 µL of NM2+Beads solution to each corresponding sample and control.
2. Select the appropriate script on the instrument.
   - KingFisher™ Flex/MagMAX™ Express-96: **NM_LSI_RRC96**
   - KingFisher™ mL: **NM_LSI_15prep**
3. 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.
4. 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 appropriate film.
KingFisher™ mL	Remove the tube strip at position 5, then transfer the purified nucleic acid to new microcentrifuge tubes.

   a. Discard the other plastic consumables used in the run (plates, strips, combs).

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

Purify RNA using the MagMAX™-96 Viral RNA Isolation Kit (automated method)

The following protocol can be used with the MagMAX™ Express-96 instrument.

Before first use of the kit

Prepare the following reagents according to the recommendations of the supplier.

1. For Wash Solution 1—Add the required volume of 100% isopropanol to the bottle of Wash Solution 1 Concentrate.
2. For Wash Solution 2—Add the required volume of 96–100% ethanol to the bottle of Wash Solution 2 Concentrate.

1 Prepare TL lysis buffer

1. Combine the following components for the required number of samples plus 10% overage, then vortex briefly to mix.

   Note: The Carrier RNA can become viscous after thawing. If needed, warm the solution at 37°C for 10–15 minutes, vortex vigorously, then centrifuge before pipetting.

Component	Volume per sample
Lysis/Binding Solution Concentrate	70 µL
Carrier RNA	1 µL
Total Lysis/Binding Solution Concentrate + Carrier RNA	**71 µL**

2. Add 100% isopropanol to the Lysis/Binding Solution Concentrate + Carrier RNA, then vortex thoroughly to mix.

Component	Volume per sample
Lysis/Binding Solution Concentrate + Carrier RNA	71 µL
100% isopropanol	70 µL
Total TL lysis buffer	**141 µL**

3. Keep the TL lysis buffer at room temperature until use.

Once reconstituted, the TL lysis buffer is stable for 1 month at room temperature. Do not store the TL lysis buffer at 2–8°C as the carrier RNA may precipitate. If the TL lysis buffer is stored at 2–8°C by mistake, incubate at 37°C for 10–15 minutes, then mix thoroughly before use.

2 Prepare Bead Solution

Prepare Bead Solution at the time of use. Do not mix in advance.

1. Vortex the RNA Binding Beads thoroughly to ensure that the beads are fully resuspended.
2. Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
RNA Binding Beads	10 µL
Lysis/Binding Enhancer	10 µL
Total Bead Solution	**20 µL**

3. Mix the Bead Solution by inversion until the solution is homogeneous, then place on ice until use.

Discard the Bead Solution after use.

3 Combine samples with the Bead Solution

Add the following components to the appropriate wells in the sample plate (Deep Well plate).

Component	Volume per test sample	Volume per mock sample
Prepared sample	50 µL of whole blood or serum	—
Bead Solution	20 µL	20 µL

4 Set up the processing plates or tube strips

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

Position[1]	Plate type	Reagent	Volume per well
2	Standard	Wash Solution 1	170 µL
3	Standard	Wash Solution 1	170 µL
4	Standard	Wash Solution 2	170 µL
5	Standard	Wash Solution 2	170 µL
6	Standard	Elution Buffer	50 µL
7	Standard	Place a tip comb in the plate.	

[1] Position on the instrument.

5 Process samples on instrument

1. Add 140 µL of the TL lysis buffer to each sample and control (position A of the strip or plate 1 are recommended).
2. Select the **AM_LSI_Express** script on the instrument:
3. 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.
4. At the end of the run, when prompted by the instrument, remove the plate at position 6, then transfer the purified RNA to the elution storage plate (provided with the kit) or new microcentrifuge tubes.

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

Purify RNA using the QIAamp™ Viral RNA Mini Kit (manual method)

Before first use of the kit

- Reconstitute the AVL+Carrier Buffer—Follow the recommendations of the supplier.
- 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. Combine the following components in the order indicated, then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock sample
Prepared sample	100 µL of whole blood or serum	—
DNase/RNase-free water	—	100 µL
AVL+Carrier Buffer	560 µL	560 µL

2. Vortex for 15 seconds.
3. Incubate at room temperature for 10 minutes.
4. Add 560 µL of 96–100% ethanol to each sample, vortex for 15 seconds, then briefly centrifuge to collect the contents.

2 Bind the RNA to the column

1. Insert a QIAamp™ Viral RNA Mini Kit column into a collection tube, then transfer 630 µL of the sample lysate to the column.
2. Cap the column, then centrifuge the assembly at 10,000 × *g* for 1 minute.
3. Discard the collection tube, then place the column on a new collection tube.
4. Transfer the remaining sample lysate volume to the column, cap the column, then centrifuge at 10,000 × *g* for 1 minute.
5. Discard the collection tube, then place the column on a new collection tube.

3 Wash, then elute the RNA

1. Add 500 µL of AW1 Buffer to each column, cap the column, then centrifuge at 6,000 × *g* for 1 minute.
2. Discard the collection tube, then place the column on a new collection tube.
3. Add 500 µL of AW2 Buffer to each column, cap the column, then centrifuge at 10,000 × *g* for 1 minute.
4. Discard the collection tube, then place the column on a new 2-mL collection tube.
5. Centrifuge at 10,000 × *g* for 3 minutes to dry the membrane.
6. Discard the collection tube.
7. Place the column on a new 1.5-mL microtube, then add 40 µL of AVE Buffer.
8. Cap the column, then incubate at room temperature for 1 minute.
9. Centrifuge at 6,000 × *g* for 2 minutes, then discard the column.
   The purified RNA is in the microtube.

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

Purify RNA using the NucleoSpin™ RNA Virus kit (manual method)

Before first use of the kit

- Reconstitute the RAV1+Carrier Buffer—Follow the recommendations of the supplier.
- Reconstitute the RAV3 Buffer—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. Combine the following components in the order indicated, then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock sample
Prepared sample	100 µL of whole blood or serum	—
DNase/RNase-free water	—	100 µL
RAV1+Carrier Buffer	560 µL	560 µL

2. Vortex for 15 seconds.

1	Lyse, then homogenize the samples *(continued)*	3. Incubate at room temperature for 10 minutes. **Note:** For coagulated blood, incubate at 70°C for 10 minutes. 4. Add 560 µL of 96–100% ethanol to each sample, vortex for 15 seconds, then briefly centrifuge to collect the contents.
2	**Bind the RNA to the column**	1. Insert a NucleoSpin™ RNA Virus kit column into a collection tube, then transfer 630 µL of the sample lysate to the column. 2. Cap the column, then centrifuge the assembly at 10,000 × *g* for 1 minute. 3. Discard the collection tube, then place the column on a new collection tube. 4. Transfer the remaining sample lysate volume to the column, cap the column, then centrifuge at 10,000 × *g* for 1 minute. 5. Discard the collection tube, then place the column on a new collection tube.
3	**Wash, then elute the RNA**	1. Add 500 µL of RAW Buffer to each column, cap the column, then centrifuge at 10,000 × *g* for 1 minute. 2. Discard the collection tube, then place the column on a new collection tube. 3. Add 630 µL of RAV3 Buffer to each column, cap the column, then centrifuge at 10,000 × *g* for 1 minute. 4. Discard the collection tube, then place the column on a new 2-mL collection tube. 5. Centrifuge at 10,000 × *g* for 3 minutes to dry the membrane. 6. Discard the collection tube. 7. Place the column on a new 1.5-mL microtube, then add 50 µL of DNase/RNase-free water. 8. Cap the column, then incubate at room temperature for 1 minute. 9. Centrifuge at 10,000 × *g* for 1 minute, then discard the column. The purified RNA is in the microtube. Store the purified RNA at 2–8°C for immediate use or below –16°C for long-term storage.

Purify RNA using the NucleoSpin™ 8 Virus/NucleoSpin™ 96 Virus kit (manual method)

Before first use of the kit

- Reconstitute the RAV1+Carrier Buffer—Follow the recommendations of the supplier.
- Reconstitute the RAV3 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. Combine the following components in a lysis plate (MN Round-Well Block) or lysis strip (rack of tube strips), then immediately proceed to the next step.

Component	Volume per test sample	Volume per mock sample
Prepared sample	100 µL of whole blood or serum	—
DNase/RNase-free water	—	100 µL
RAV1+Carrier Buffer	400 µL	400 µL
Proteinase K	20 µL	20 µL

2. Pipet up and down 4–5 times to mix, then seal the plate with adhesive film.
3. Incubate at 70°C for 10 minutes.
4. Centrifuge the lysates briefly to bring down condensation.
5. Add 400 µL of 96–100% ethanol to each lysate, then pipet up and down 4–5 times to mix.

2 Bind the RNA to the column

1. Place a NucleoSpin™ Virus Binding Plate (extraction plate) or NucleoSpin™ Virus 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. Seal the extraction plate/strip with adhesive film, then centrifuge the assembly at 5,600 × *g* for 2 minutes.
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 RNA

Preheat an aliquot of DNase/RNase-free water to 70°C.

1. Add 500 µL of RAW Buffer to each well, seal with adhesive film, then centrifuge at 5,600 × *g* for 2 minutes.
2. Discard the MN Square-Well Block, then place the extraction plate/strip on a new MN Square-Well Block.
3. Add 700 µL of RAV3 Buffer to each well, seal with adhesive film, then centrifuge at 5,600 × *g* for 2 minutes.
4. Discard the MN Square-Well Block, then place the extraction plate/strip on a new MN Square-Well Block.
5. Add 700 µL of RAV3 Buffer to each well, seal with adhesive film, then centrifuge at 5,600 × *g* for 15 minutes.
6. Discard the MN Square-Well Block.
7. Place the extraction plate/strip on an elution plate or strip, then add 80 µL of DNase/RNase-free water (preheated to 70°C) to each well.
8. Seal the extraction plate/strip with adhesive film, then incubate at room temperature for 1–2 minutes.
9. Centrifuge at 5,600 × *g* for 2 minutes, then discard the extraction plate/strip. The purified RNA is in the elution plate/strip.

Store the purified RNA 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 Blood storage guidelines applicable to France

Blood storage guidelines applicable to France

The French National Reference Laboratory suggests the following instructions:

Collect blood in EDTA tubes. Following collection, maintain at 2°C to 8°C until use and for a maximum of 10 days after sampling. After use or after the 10-day period, freeze below –16°C for storage up to 1 year or below –70°C for storage longer than 1 year.

Appendix B Purify nucleic acid using 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 deep-well 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 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 8).

   Note: For rapid processing of samples, select one of the following express scripts on the instrument.
   - KingFisher™ Duo Prime: **MagMAX_CORE_DUO_Express.bdz**
   - KingFisher™ mL: **MagMAX_CORE_mL_Express.bdz**

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.
- For samples processed with an express script, perform real-time RT-PCR using the express thermal-cycling method (see the *VetMAX™ EHDV Kit Instructions for Use* (Pub. No. MAN0008223)).

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Revision	Date	Description
D.0	15 November 2023	The note about Express scripts was removed (see "Recommended workflows for use with the MagMAX™ CORE Nucleic Acid Purification Kit" on page 5).
C.0	6 September 2023	• The MagMAX™ CORE Nucleic Acid Purification Kit was added (see "Purify nucleic acid using the MagMAX™ CORE Nucleic Acid Purification Kit" on page 5). • Made minor wording and formatting updates for consistency with related documents.
B.0	19 February 2018	• Updated to the current document template, with associated updates to the warranty, trademarks, and logos. • Added Appendix A, "Blood storage guidelines applicable to France".
A.0	20 June 2014	Baseline for revision history