VetMAX™ BTV8 IAH Typing Kit

Nucleic acid purification protocols

Catalog Number BTV8GIAH50

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IMPORTANT! In France, follow Appendix B, "Blood storage guidelines applicable to France".

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! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at thermofisher.com. Wear appropriate protective eyewear, clothing, and gloves.

Species	Isolation of nucleic acid from matrices	Test type
Bovine Small ruminants (sheep, goats)	EDTA-treated whole blood Spleen or aborted fetus (spleen, liver, heart)	Individual

Contents of this manual

This manual describes bluetongue virus (BTV) viral RNA purification protocols compatible with the **Applied Biosystems™ VetMAX™ BTV8 IAH Typing Kit**.

Sample selection

Sample matrix	Type of analysis	Quantity required and sampling equipment
Whole blood	Individual	50–200 µL of whole blood collected in EDTA tubes[1]
Organ sample	Individual	1 g of spleen, liver, or heart

[1] Quantity required depends on the extraction protocol used.

Sample storage

Ensure the quality of the samples before starting the extraction protocol.

Whole blood

The blood must always be collected in EDTA tubes. Following collection, maintain at **2°C to 8°C** until use and for a maximum of **4 days after sampling** (do not freeze). After use or after the 4 days, freeze **below –16°C for storage up to 1 year** or **below –70°C for storage longer than 1 year.**

Note: In France, store blood samples according to Appendix B, "Blood storage guidelines applicable to France".

Organ sample

After sampling, maintain the samples **at 2°C to 8°C** until use **(up to 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**.

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

Materials and reagents required for the preparation of samples for analysis

- Class II microbiological safety cabinet (MSC)
- Precision micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filter tips
- Vortex or equivalent
- Centrifuge for 1.5 mL and 2 mL microtubes
- Precision balance
- 1.5 mL and 2 mL DNase/RNase-free microtubes
- A laboratory mill for the mechanical grinding of organ samples
- 1X PBS buffer or minimum essential medium (MEM)
- DNase/RNase-free water

Kits, reagents, and equipment for extraction and purification of RNA from samples

All equipment and reagents that are required to prepare samples for analysis are likely to be used for this step. In addition, ensure that the following are available:

- 96–100% ethanol or 80% ethanol according to the extraction performed
- Automated magnetic bead extraction:
  - MagMAX™ CORE Nucleic Acid Purification Kit (Cat. No. A32700 or A32702)
  - MagVet™ Universal Isolation Kit (Cat. No. MV384)
  - MagMAX™-96 Viral RNA Isolation Kit (Cat. No. AM1836) + 100% isopropanol
  - Magnetic purification instrument: information available on request from Technical Support

- Manual extraction on columns of silica in individual format:
  - QIAamp™ Viral RNA Mini Kit (QIAGEN™) or NucleoSpin™ RNA Virus kit (MACHEREY-NAGEL)
  - Centrifuge for microtubes
- Manual extraction on columns of silica in plate format:
  - NucleoSpin™ 8 / 96 Virus kit (MACHEREY-NAGEL)
  - Centrifuge for plates

Recommended RNA extraction kits

	Recommended extraction kits
Whole blood	MagMAX™ CORE Nucleic Acid Purification Kit
	MagVet™ Universal Isolation Kit
	MagMAX™-96 Viral RNA Isolation Kit[1]
	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

[1] This protocol has not been validated by the French National Reference Laboratory for BTV.

Preparation of samples before extraction

Whole blood collected in EDTA tubes

Collect whole blood in EDTA tubes, then gently invert the tubes several times to mix the contents before testing. After mixing, **use the sample directly** without additional preparation before the extraction. The extraction requires **50–200 µL of blood**, depending on the method.

Prepare the organ sample

1. Finely dissect the organ piece in a sterile Petri dish using sterile forceps and a scalpel.
2. Weigh 1 g of the dissected organ in a sample jar using a precision balance.
3. Add 10 mL of physiological saline or buffered solution (e.g., 1X PBS).
4. Transfer everything to a mixer and grind for approximately 10–15 seconds.
5. Transfer the ground sample to a new sample jar.
6. Transfer 1 mL of the ground sample to a 2-mL microtube.
7. Centrifuge for 2 minutes at 1000 × *g* at 4°C.
8. Use **100 µL of supernatant** to perform the extraction.

Extraction with the MagMAX™ CORE Nucleic Acid Purification Kit

This protocol is intended for extraction only from whole blood in EDTA tubes.

Recommended workflows

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

- Multisample Simple Workflow
- Simple Workflow
- Whole Blood Workflow

The Multisample Simple, Simple and Whole Blood workflows differ in the order and amount 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 5).

Table 1 Recommended thermal-cycling method for whole blood samples

Sample matrix	Recommended extraction workflow	Thermal-cycling method on the PCR instrument	
		Recommended	Alternative
Whole blood	Simple workflow	Standard program	Express program
	Whole Blood workflow	Standard program	N/A

Workflow: Multisample Simple

Start with whole blood samples

▼

Follow the appropriate procedure based on your instrument:

KingFisher™ Flex or MagMAX™ Express-96

Set up the processing plates (page 6)

▼

Prepare samples for processing using the Multisample Simple Workflow (page 7)

Prepare Lysis/Binding/Bead Mix (page 7)

Prepare the sample (page 7)

Combine the sample with PK, then add Lysis/Binding/Bead Mix (page 7)

▼

Process samples on the instrument (page 10)

KingFisher™ Duo Prime or KingFisher™ mL

Prepare samples for processing using the Multisample Simple Workflow (page 7)

Prepare Lysis/Binding/Bead Mix (page 7)

Prepare the sample (page 7)

Combine the sample with PK, then add Lysis/Binding/Bead Mix (page 7)

▼

Set up the processing plates (page 10)

▼

Process samples on the instrument (page 10)

Workflow: Simple

Start with whole blood samples

▼

Follow the appropriate procedure based on your instrument:

KingFisher™ Flex or MagMAX™ Express-96

Set up the processing plates (page 6)

▼

Prepare samples for processing using the Simple Workflow (page 7)

Prepare Bead/PK Mix (page 7)

Prepare Lysis/Binding Mix (page 8)

Prepare the sample (page 8)

Combine the sample with Bead/PK Mix, then add the Lysis/Binding Mix (page 8)

▼

Process samples on the instrument (page 10)

KingFisher™ Duo Prime or KingFisher™ mL

Prepare samples for processing using the Simple Workflow (page 7)

Prepare Bead/PK Mix (page 7)

Prepare Lysis/Binding Mix (page 8)

Prepare the sample (page 8)

Combine the sample with Bead/PK Mix, then add the Lysis/Binding Mix (page 8)

▼

Set up the processing plates (page 10)

▼

Process samples on the instrument (page 10)

Workflow: Whole Blood

Start with whole blood samples

▼

Follow the appropriate procedure based on your instrument:

KingFisher™ Flex or MagMAX™ Express-96	KingFisher™ Duo Prime or KingFisher™ mL
Set up the processing plates (page 6)	**Prepare samples for processing using the Whole Blood Workflow (page 8)**
▼	Prepare PK/PBS Mix (page 8)
Prepare samples for processing using the Whole Blood Workflow (page 8)	Prepare Lysis/Binding/Bead Mix (page 9)
Prepare PK/PBS Mix (page 8)	Prepare the sample (page 9)
Prepare Lysis/Binding/Bead Mix (page 9)	Combine the sample with PK/PBS Mix, then add the Lysis/Binding/Bead Mix (page 9)
Prepare the sample (page 9)	▼
Combine the sample with PK/PBS Mix, then add the Lysis/Binding/Bead Mix (page 9)	**Set up the processing plates (page 10)**
▼	▼
Process samples on the instrument (page 10)	**Process samples on the instrument (page 10)**

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

(*Optional*) Determine the maximum plate shaker setting

If a plate shaker is used, 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. Right-click the appropriate file to download the latest version of the `MagMAX_CORE` script for your instrument.

Table 2 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 3 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 for installing the script.

Set up the processing plates (KingFisher™ Flex and MagMAX™ Express-96 instruments)

IMPORTANT! If you are using the KingFisher™ Duo Prime or KingFisher™ mL instrument, do not set up the processing plates or tube strips before preparing the samples.

1. Set up the processing plates on the 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.

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 "Prepare samples for processing using the Multisample Simple Workflow" on page 7.
- **Simple Workflow**—See "Prepare samples for processing using the Simple Workflow" on page 7.
- **Whole Blood Workflow**—See "Prepare samples for processing using the Whole Blood Workflow" on page 8

Prepare samples for processing using the Multisample Simple Workflow

1 Prepare Lysis/Binding/Bead Mix

1. Combine the following components, in the order indicated, 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**

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

2 Prepare the sample

Prepare samples and controls as described.

Sample type	Action
Whole blood	Proceed with 200 µL of sample.
NCS	—

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

1. Add 10 µL of MagMAX™ CORE Proteinase K to the required wells in the plate or tube strip.
2. Transfer each prepared sample or control to a well with MagMAX™ CORE Proteinase K.
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 "(Optional) Determine the maximum plate shaker setting" on page 5).
   - **By pipetting:** pipet up and down 3 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 10.

   Note: If you are using the KingFisher™ Duo Prime or KingFisher™ mL instrument, proceed to "Set up the processing plates or tube strips (KingFisher™ Duo Prime and KingFisher™ mL instruments)" on page 10.

Prepare samples for processing using the Simple Workflow

1 Prepare Bead/PK Mix

We recommend that you prepare new Bead/PK Mix for each processing run. If necessary, you can store Bead/PK Mix at 4°C for up to 1 week.

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

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

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

(*Optional*) Store Lysis/Binding 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 200 µL of sample.
NCS	—

4 Combine the sample with Bead/PK Mix, then add the 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 required wells in the plate or tube strip.
2. Transfer each prepared sample to a well with Bead/PK Mix.
3. Mix the sample with Bead/PK Mix for 2 minutes at room temperature according to your mixing method.
   - **Using a plate shaker**: shake vigorously for 2 minutes (see "(Optional) Determine the maximum plate shaker setting" on page 5).
   - **By pipetting:** pipet up and down 3 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 strip.
5. Immediately proceed to "Process samples on the instrument" on page 10.

   Note: If you are using the KingFisher™ Duo Prime or KingFisher™ mL instrument, proceed to "Set up the processing plates or tube strips (KingFisher™ Duo Prime and KingFisher™ mL instruments)" on page 10.

Prepare samples for processing using the Whole Blood Workflow

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 the Lysis/Binding/Bead Mix to each sample-containing well or tube strip.
6. Immediately proceed to “Process samples on the instrument” on page 10.

   Note: If you are using the KingFisher™ Duo Prime or KingFisher™ mL instrument, proceed to “Set up the processing plates or tube strips (KingFisher™ Duo Prime and KingFisher™ mL instruments)” on page 10.

Set up the processing plates or tube strips (KingFisher™ Duo Prime and KingFisher™ mL instruments)

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 tube strips required by this instrument.

1. Add each reagent to the indicated positions, according to your instrument.

   Load the Tip Comb and all of the plates or tube strips at the same time. The instrument does not prompt you to load items individually.

Table 4 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 5 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.	

2. Immediately proceed to “Process samples on the instrument” on page 10.

Process samples on the instrument

1. Select the appropriate script on the instrument (see “Download and install the script” on page 5).

   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**
   - KingFisher™ Duo Prime: **MagMAX_CORE_DUO_Express.bdz**
   - KingFisher™ mL: **MagMAX_CORE_mL_Express.bdz**

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.

Extraction with the MagVet™ Universal Isolation Kit

Notes

This extraction protocol can be used with the KingFisher™ mL, KingFisher™ 96, and KingFisher™ Flex instruments. Only the number of samples that are processed differs.

This protocol is only appropriate for extraction from whole blood in EDTA tubes.

Before first use of the kit

Prepare the **NM1** lysis solution—Add 100 mL of **N1** solution to the bottle containing the M1 solution (25 mL), then vortex well.

Store the NM1 lysis solution (125 mL) at room temperature for up to one year.

Before each use of the kit

- Prepare the **NM2+Beads** solution—For each reaction, combine 20 µL of **NM_LSI_Beads** and 600 µL of **NM2 binding buffer**, then vortex the solution thoroughly to ensure that the beads are fully resuspended.
  After use, discard the **NM2+Beads** solution.
- Prepare and label microtubes, strips, and plates for the required number of samples including negative and positive controls.

Protocol

If you are using the KingFisher™ mL instrument, remove the tube strip tray from the instrument, then load the extraction strips on the tray before adding the buffers.

If you are using the KingFisher™ 96 or KingFisher™ Flex instrument, buffers are added directly to the plates on the bench.

1. Set up the processing plate or strip on a laboratory bench, according to the following table.

Position of the strip or plate	Components	Sample for analysis	NCS
A / 1	Lysis solution	250 µL of NM1	250 µL of NM1
	Test sample	100 µL of blood	—
B / 2	Wash solution 1	600 µL of NM3	600 µL of NM3
C / 3	Wash solution 2	600 µL of NM4	600 µL of NM4
D / 4	Wash solution 3	600 µL of 80% ethanol	600 µL of 80% ethanol
E / 5	Elution buffer	80 µL of NM6	80 µL of NM6

2. Vortex the **NM2+Beads** solution thoroughly to resuspend the beads, then transfer **620 µL** of the **NM2+Beads** solution **(or 600 µL of NM2 and 20 µL of beads) to each sample** in **position A** of the strip or **plate 1**, depending on the instrument used.
3. Place the tip comb on the magnetic rods.
4. Load the strips or plates in the instrument.
5. Select the appropriate script on the instrument:
   - Script **NM_LSI_15prep** for the KingFisher™ mL instrument
   - Script **NM_LSI_RRC96** for the KingFisher™ 96 and KingFisher™ Flex instruments
6. Start the run.
7. After the run, prepare the purified nucleic acid for storage.
   a. For the KingFisher™ mL instrument, transfer the **purified nucleic acid** in **position E of the extraction strip** to the appropriate microtubes.
   b. For the KingFisher™ 96 and KingFisher™ Flex instruments, cover the elution plate (**plate 5**) with adhesive film.
8. Discard all plastics that are used for the extraction.

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

Extraction with the MagMAX™-96 Viral RNA Isolation Kit

This protocol has not been validated by the French National Reference Laboratory for BTV.

Notes

This extraction protocol can be used with the MagMAX™ Express-24 Magnetic Particle Processor.

This protocol is only appropriate for extraction from whole blood in EDTA tubes.

Before first use of the kit

- Prepare the **TL** lysis buffer according to the following table:

	For 1 sample	For N samples[1]
Lysis/Binding Solution Concentrate	70 µL	N × 70 µL
Carrier RNA[2]	1 µL	N × 1 µL
Vortex briefly		
Add 100% isopropanol	70 µL	N × 70 µL
Vortex		

[1] We recommend preparing an additional reaction with respect to the total number of extractions to be carried out (samples plus controls).
[2] The carrier RNA can become viscous after thawing. If it is difficult to pipette, **incubate the tube at 37°C for 10 to 15 minutes,** vortex vigorously, then centrifuge.

Store the prepared TL lysis buffer at room temperature for up to one month.

Note: Do not store the TL lysis buffer at 2–8°C as the carrier RNA can precipitate. If precipitation occurs, incubate the TL lysis buffer at 37°C for 10 to 15 minutes, then mix thoroughly before use.

- Prepare Wash solution 1—Add the required volume of 100% isopropanol to the Wash Solution 1 Concentrate according to the manufacturer's recommendations.
- Prepare Wash solution 2—Add the required volume of 96–100% ethanol to the Wash Solution 2 Concentrate according to the manufacturer's recommendations.

Before each use of the kit

- Prepare the **Mix Beads** solution—For each reaction, combine 10 µL of **RNA Binding Beads** and 10 µL of **Lysis/Binding Enhancer**, then mix the solution by gentle agitation to ensure that the beads are fully resuspended.
  Store the **Mix Beads** solution at 2–8°C for immediate use. After use, discard the **Mix Beads** solution.
- Prepare and label microtubes and plates for the required number of samples including negative and positive controls.

Protocol

1. Set up the processing plate on a laboratory bench, according to the following table.

Plate line	Components	Sample for analysis	NCS
A	Magnetic beads	20 µL of Mix Beads	20 µL of Mix Beads
	Test sample	50 µL of blood	—
B	Wash solution 1	170 µL wash buffer 1	170 µL wash buffer 1
C	Wash solution 1	170 µL wash buffer 1	170 µL wash buffer 1
D	Wash solution 2	170 µL wash buffer 2	170 µL wash buffer 2
E	Wash solution 2	170 µL wash buffer 2	170 µL wash buffer 2
F	Elution buffer	50 µL elution buffer	50 µL elution buffer

2. Transfer **140 µL** of the **TL** lysis buffer **to each sample** in **line A** of the plate.
3. Place the tip comb on the magnetic rods.
4. Load the plates in the instrument.
5. Select the script **AM_LSI_Express** on the MagMAX™ Express-24 Magnetic Particle Processor.
6. Start the run.
7. After the run, transfer the **purified nucleic acid** in **line F of the extraction plate** to the elution storage plate provided in the kit, or to the appropriate microtubes.
8. Discard all plastics that are used for the extraction.

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

Extraction with the QIAamp™ Viral RNA Mini Kit

Before starting

- Prepare the **AVL+Carrier** buffer according to the manufacturer's recommendations.
- Prepare the **AW1** and **AW2** buffers—Add the required quantity of 96–100% ethanol according to the manufacturer's recommendations before use.
- Prepare and label microtubes and columns for the required number of samples including negative and positive controls.

Protocol

1. Combine the following components in 1.5-mL microtubes according to the following table:

	Sample for analysis	NCS
Lysis solution	560 µL of AVL+Carrier	560 µL of AVL+Carrier
Test sample	100 µL of sample	100 µL of DNase/RNase-free water

2. Vortex immediately for **15 seconds.**
3. Incubate at room temperature for **10 minutes**.
4. Add **560 µL of 96–100% ethanol** to each tube, vortex immediately for 15 seconds, then centrifuge rapidly before opening the tube.

   The **sample lysate** is produced.
5. Select and label a mini column from the QIAamp™ Viral RNA Mini Kit.
6. Transfer **630 µL of the sample lysate** to the column, cap the column, centrifuge at 10,000 × *g* for 1 minute, discard the collection tube, then **proceed with the column**.
7. Transfer **the remainder of the sample lysate** to the same column, cap the column, centrifuge at 10,000 × *g* for 1 minute, discard the collection tube, then **proceed with the column**.
8. Add **500 µL** of **AW1** buffer (see "Before starting" on page 13) to each column, cap the column, centrifuge at 6,000 × *g* for 1 minute, discard the collection tube, then **proceed with the column**.
9. Add **500 µL** of **AW2** buffer (see "Before starting" on page 13) to each column, cap the column, centrifuge at 10,000 × *g* for 1 minute, discard the collection tube, then **proceed with the column**.
10. Place the column in a clean 2-mL collection tube, centrifuge at 10,000 × *g* for 3 minutes to dry the membrane, discard the collection tube, then **proceed with the column**.
11. Place the column in a clean **1.5-mL microtube**, add **40 µL** of **AVE** buffer, then cap the microtube.
12. Incubate at room temperature for 1 minute.
13. Centrifuge the column-microtube assembly at 6,000 × *g* for 2 minutes, then discard the column.

    The purified nucleic acid is in the microtube.

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

Extraction with the NucleoSpin™ RNA Virus kit

Before starting

- Prepare the **RAV1+Carrier** buffer according to the manufacturer's recommendations.
- Prepare the **RAV3** buffer—Add the required quantity of 96–100% ethanol according to the manufacturer's recommendations before use.
- Prepare and label microtubes and columns for the required number of samples including negative and positive controls.

Protocol

1. Combine the following components in 1.5-mL microtubes according to the following table:

	Sample for analysis	NCS
Lysis solution	560 µL of RAV1+Carrier	560 µL of RAV1+Carrier
Test sample	100 µL of sample	100 µL of DNase/RNase-free water

2. Vortex immediately for **15 seconds.**
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 tube, vortex immediately for 15 seconds, then centrifuge rapidly before opening the tube.

   The **sample lysate** is produced.
5. Select and label a mini column from the NucleoSpin™ RNA Virus kit.
6. Transfer **630 µL of the sample lysate** to the column, cap the column, centrifuge at 10,000 × *g* for 1 minute, discard the collection tube, then **proceed with the column**.
7. Transfer **the remainder of the sample lysate** to the same column, cap the column, centrifuge at 10,000 × *g* for 1 minute, discard the collection tube, then **proceed with the column**.
8. Add **500 µL** of **RAW** buffer to each column, cap the column, centrifuge at 10,000 × *g* for 1 minute, discard the collection tube, then **proceed with the column**.
9. Add **630 µL** of **RAV3** buffer (see "Before starting" on page 13) to each column, cap the column, centrifuge at 10,000 × *g* for 1 minute, discard the collection tube, then **proceed with the column**.
10. Place the column in a clean 2-mL collection tube, centrifuge at 10,000 × *g* for 3 minutes to dry the membrane, discard the collection tube, then **proceed with the column**.
11. Place the column in a clean **1.5-mL microtube**, add **50 µL of DNase/RNase-free water**, then cap the microtube.
12. Incubate at room temperature for 1 minute.
13. Centrifuge the column-microtube assembly at 10,000 × *g* for 1 minute, then discard the column.

    The purified nucleic acid is in the microtube.

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

Extraction with the NucleoSpin™ 8 / 96 Virus kit

Notes

Extractions with the NucleoSpin™ 8 Virus and NucleoSpin™ 96 Virus kits are similar, only the format of the columns differs, bars of 8 columns or a plate of 96 columns.

Before starting

- Prepare the **RAV1+Carrier** buffer according to the manufacturer's recommendations.
- Prepare the **RAV3** buffer—Add the required quantity of 96–100% ethanol according to the manufacturer's recommendations before use.
- Prepare the proteinase K—Add the required quantity of PB buffer according to the manufacturer's recommendations before use.
- Prepare and label lysis strips or plates and silica columns in strip or plate format for the required number of samples including negative and positive controls.

Protocol

1. Combine the following components in the lysis strips (rack of tube strips) or lysis plates (MN Round-Well Block) according to the following table:

	Sample for analysis	NCS
Lysis solution	400 µL of RAV1+Carrier	400 µL of RAV1+Carrier
	20 µL of Proteinase K	20 µL of Proteinase K
Test sample	100 µL of sample	100 µL of DNase/RNase-free water

2. **Mix** by pipetting up and down 4 to 5 times, then cap securely.

3. Incubate at **70°C for 10 minutes**, then centrifuge rapidly before opening.

4. Add **400 µL of 96–100% ethanol** to an MN Square-Well Block, transfer the lysate that is obtained in the previous step to the 96–100% ethanol, then **mix** by pipetting up and down 4 to 5 times.

   The **sample lysate** is produced.

5. Select and label columns from the NucleoSpin™ 8 / 96 Virus kit (blue columns), in strip format (NucleoSpin™ Virus Binding Strips) or plate format (NucleoSpin™ Virus Binding Plate), then place them on an MN Square-Well Block.

6. Using a pipette, transfer **all the sample lysate** to the columns, close with adhesive film, centrifuge at 5,600 × *g* for 2 minutes, then **transfer the columns** to another MN Square-Well Block or empty wells.

7. Add **500 µL** of **RAW** buffer to each column, close with adhesive film, centrifuge at 5,600 × *g* for 2 minutes, then **transfer the columns** to another MN Square-Well Block or empty wells.

8. Add **700 µL** of **RAV3** buffer (see "Before starting" on page 14) to each column, close with adhesive film, centrifuge at 5,600 × *g* for 2 minutes, then transfer the columns to another MN Square-Well Block or empty wells.

9. Add **700 µL** of **RAV3** buffer (see "Before starting" on page 14) to each column, close with adhesive film, then centrifuge at 5,600 × *g* for 15 minutes.

10. **Transfer the columns** to elution strips or an elution plate, add **80 µL of DNase/RNase-free water preheated to 70°C**, then close with adhesive film.

11. Incubate at room temperature for 1 to 2 minutes.

12. Centrifuge at 5,600 × *g* for 2 minutes, discard the columns, then **cap and keep the elution tubes.**

    The purified nucleic acid is in the elution tubes.

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

Appendix A Retesting procedure for samples with invalid real-time RT-PCR results

Retest samples with invalid real-time RT-PCR results

If an invalid result is obtained for a sample, we recommend performing one of the following procedures according to the quality of the RNA eluate.

For...	Do this...
Easy-to-pipet (low viscosity) eluates	a. Dilute the RNA eluate 1:5 in 1X TE buffer. b. Denature the diluted RNA for 3 minutes at 92°C to 98°C. c. Repeat the real-time RT-PCR procedure with 5 µL of the denatured RNA, then interpret the results as indicated. • If the real-time RT-PCR result is positive or negative for BTV, and the IPC result is acceptable (C_t IPC < 35), the result is validated. • If the real-time RT-PCR result remains invalid (C_t IPC ≥ 35), dilute the sample 1:2 in 1X PBS buffer, then repeat the nucleic acid extraction procedure.
Difficult-to-pipet (high viscosity) eluates	a. Dilute the sample 1:2 in 1X PBS buffer. b. Repeat the nucleic acid extraction procedure. c. Denature the RNA eluate for 3 minutes at 92°C to 98°C. d. Repeat the real-time RT-PCR procedure with 5 µL of the denatured RNA, then interpret the results as indicated. • If the real-time RT-PCR result is positive or negative for BTV, and the IPC result is acceptable (C_t IPC < 35), the result is validated. • If the real-time RT-PCR result remains invalid (C_t IPC ≥ 35), repeat the analysis on a new sample.

Appendix B 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.

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Revision history: Pub. No. MAN0017850 D

Revision	Date	Description
D	13 January 2025	• A table was added that lists recommended amplification protocols based on the extraction protocol. • Statements were removed that linked express extraction scripts to express thermal-cycling methods.
C.0	6 September 2023	The Whole Blood Workflow for whole blood protocol was added (see "Prepare samples for processing using the Whole Blood Workflow" on page 8).
B.0	19 February 2021	• Added express scripts to the MagMAX™ CORE Nucleic Acid Purification Kit protocol (for use with the real-time RT-PCR express thermal-cycling method). • Added Appendix A, "Retesting procedure for samples with invalid real-time RT-PCR results". • Added note to indicate that the MagMAX™-96 Viral RNA Isolation Kit has not been validated by the French National Reference Laboratory for BTV. • Made minor wording and formatting updates for consistency with related documents.
A.0	19 September 2018	Baseline for revision history.