PRODUCT INFORMATION

Thermo Scientific
MagJET Viral DNA and RNA Kit

#K2781, #K2782

Read Storage information (p. 4) upon receipt and store kit components appropriately!

www.thermoscientific.com/onebio

#K2781, #K2782

Lot 00000000

Expiry Date 00.0000

CERTIFICATE OF ANALYSIS

Thermo Scientific™ MagJET™ Viral DNA and RNA Kit has been tested by isolating DNA and RNA from 200 µL of spiked human plasma. Yield of nucleic acids was evaluated by RT-qPCR and qPCR, and compared with spiked DNA (linearized plasmid DNA, 10^5 copies/sample) and RNA (RNA transcript, 10^6 copies/sample) copy number.

Quality authorized by: **Jurgita Žilinskienė**

Rev.1 h

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COMPONENTS OF THE KIT

MagJET Viral DNA and RNA Kit	#K2781 96 preps	#K2782 384 preps
Lysis Buffer for MagJET Viral Kit	40 mL	155 mL
Proteinase K	5.2 mL	21 mL
Carrier RNA	1 vials	4 vials
MagJET Magnetic Beads	2 × 1.4 mL	1 × 10.6 mL
Wash Buffer 1 (conc.) for MagJET Viral Kit	45 mL	2 × 90 mL
Wash Buffer 2 (conc.) for MagJET Viral Kit	50 mL	3 × 50 mL
Water, nuclease free	30 mL	2 × 30 mL

STORAGE

When the kit is delivered, remove the Carrier RNA from the package and store in the original aluminum bag at -20°C. Proteinase K solution is stable at room temperature as long as the vial remains sealed. After being opened it should be stored at -20°C. MagJET Magnetic Beads should be stored at 4°C. Other components of the kit should be stored at room temperature (15-25°C).

DESCRIPTION

The MagJET Viral DNA and RNA Kit is designed for fast and efficient purification of viral nucleic acids from various human and animal liquid samples such as plasma, serum, saliva and urine, as well as from nasal, buccal and urogenital swabs and blood.
The kit utilizes paramagnetic bead technology enabling high yields and robust performance. High binding capacity, uniform particle size, and rapid magnetic response of MagJET magnetic beads makes the technology ideal for high throughput automatic nucleic acid purification, as well as for manual purification by low sample throughput users.
The resulting high quality viral nucleic acids are free of proteins, nucleases, and other contaminants or inhibitors. The purified DNA and RNA is suitable for direct use in qPCR, RT-qPCR and other enzymatic reactions.

PRINCIPLE

The MagJET Viral DNA and RNA Kit uses the highly efficient MagJET magnetic particle based technology for nucleic acid purification. The whole nucleic acid isolation process combines simple steps of sample lysis, DNA or RNA binding to the magnetic beads, washing and elution.

Purification protocols optimized for automated KingFisher instruments utilize high throughput magnetic bead transfer technique where magnetic beads are transferred through different reagent plates containing lysis, binding, washing and elution reagents. This enables high throughput nucleic acid purification and eliminates multiple pipetting steps.

Alternatively, protocol is provided where instead of magnetic particles, buffers and other reagents are transferred in each of the protocol steps, while magnetic beads are captured on the wall of the tube with the help of a magnetic rack. This allows the kit to be used in various throughput applications using a magnetic rack and manual or automated pipetting equipment.

IMPORTANT NOTES

- Add the indicated volume of isopropanol (100%) to concentrated **Wash Buffer 1** and indicated volume ethanol (96-100%) to concentrated **Wash Buffer 2** prior to first use:

	#K2781		#K2782	
	Wash Buffer 1	**Wash Buffer 2**	**Wash Buffer 1**	**Wash Buffer 2**
Concentrated Wash buffer	45 mL	50 mL	90 mL	50 mL
Isopropanol (100%)	**37 mL**	**-**	**74 mL**	**-**
Ethanol (96-100%)	-	**200 mL**	-	200 mL
Total volume	82 mL	250 mL	164 mL	250 mL

After preparing each solution, mark the bottle to indicate that this step has been completed.

- Check all solutions in the kit for any salt precipitation before each use. Re-dissolve any precipitates by warming the solution at 37°C, and then equilibrate to room temperature (15-25°C).
- All sample material and waste should be regarded as potentially infectious. Wear the proper protection when handling samples and waste. Avoid any skin or eye contact! Work under laminar air flow conditions if possible until samples are lysed. Disinfect all work surfaces thoroughly after the procedure. Follow proper waste disposal guidelines as recommended by local authorities.
- The following steps should be taken in order to avoid cross-contamination: always change pipette tips between liquid transfers (aerosol-barrier pipette tips are recommended), open only one tube at a time, use disposable gloves and discard if contaminated. Always use RNase-free equipment.
- Use only a freshly prepared mixture of **Carrier RNA** and **Lysis Buffer** when beginning a new extraction procedure. Use 25 µL **Carrier RNA** to 1 mL **Lysis Buffer**. Supplement Lysis Buffer with the required quantity of Carrier RNA and mix by pulse-vortexing or pipetting.
- Wear gloves when handling the Lysis Buffer and Wash Buffer 1 as these reagents contain irritants (*see* page 21 for SAFETY INFORMATION).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and sterile, nuclease-free pipette tips with aerosol barrier.
- 1.5 mL or 2.0 mL tubes, RNase free.
- Microcentrifuge.
- Thermomixer.
- Nuclease-free tubes of an appropriate size for preparing mixtures of Carrier RNA and Lysis buffer.
- Disposable gloves.
- 100% isopropanol, molecular biology grade.
- 96-100% ethanol, molecular biology grade.
- Automatic magnetic particle processor and consumables *or* Magnetic particle processing rack.
- Buffers:
  a) PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4).
  b) 0.9% NaCl solution.
  c) TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).
  d) 0.5 M EDTA.

STARTING MATERIAL HANDLING AND STORAGE

- If possible, use fresh sample material.
- Plasma and serum samples can be stored at 2-8°C for up to 24 hours, or at -20°C or -70°C for long term storage.
- Urogenital swabs can be stored at 2-8°C for up to 48 hours. For longer term storage cells should be collected by centrifugation and stored at -20°C or -70°C.
- Nasal and buccal swabs can be stored at 2-8°C for up to 48 hours.
- Urine samples can be stored at 2-8°C for up to 12 hours (with 0.5 M EDTA added to 50 mM final concentration). For longer storage cells should be collected by centrifugation and stored at -20°C or -70°C. For viral RNA purification, it is recommended to collect the cells by centrifugation immediately after sample collection.
- Do not freeze/thaw samples more than once.
- Equilibrate samples to room temperature (15-25°C) before use. Remove precipitates from plasma/serum samples by centrifugation for 5 min at 3,000 × g.

CARRIER RNA

Usage of Carrier RNA is important for efficient recovery of viral nucleic acids for two reasons. First, Carrier RNA facilitates binding of viral nucleic acids to the magnetic beads, especially when there are only a small number of viral nucleic acid molecules in the sample. Additionally, in the rare event when there are a small number of active RNase molecules, large amounts of Carrier RNA reduce the probability of viral RNA being degraded. Failing to add Carrier RNA to the Lysis Buffer, could result in reduced viral nucleic acid yield.

PREPARATION OF CARRIER RNA

Carrier RNA is provided in a dry powder packed in a moisture-impermeable aluminum bag. Prior to the first use, reconstitute the Carrier RNA by adding 600 µL of nuclease free water. Incubate freshly reconstituted Carrier RNA for 5 min at room temperature, then mix thoroughly and briefly centrifuge the vial. Use immediately or store at -20°C. Do not freeze-thaw the reconstituted Carrier RNA more than 10 times.

INTERNAL CONTROL

The presence of an internal control throughout the extraction and purification procedure may be necessary for certain assays. Please refer to the user manual provided with the downstream detection assay for further directions on how to use an internal control.

PROTOCOL SELECTION GUIDE

The MagJET Viral DNA and RNA Kit provides optimized protocols for viral DNA and RNA purification from various human and animal samples such as plasma, serum, urine, nasal and buccal swabs, saliva, urogenital swabs and blood. The kit is compatible with automated and manual sample processing, allowing low- to high-throughput nucleic acid purification workflow. The following selection guide summarizes available protocols depending on starting sample volume, throughput and sample processing type. Automation protocols are optimized for KingFisher Flex and KingFisher Duo instruments.

Note: Transfer the **Viral_NA_Flex** protocol file to the KingFisher Flex or **Viral_NA_Duo** protocol file to the KingFisher Duo instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.2 User Manual. The protocol files for MagJET Viral DNA and RNA Kit can be found on product web page on www.thermoscientific.com/onebio

Protocol selection guide:

Sample type	Sample volume	Throughput per run	KingFisher Flex Instrument	KingFisher Duo Instrument	Manual processing	MagJET purification protocol	Page
Human or animal plasma, serum	up to 200 µL	96	●	-	-	**Protocol A**	page 8
		12	-	●	-	**Protocol B**	page 10
	up to 400 µL	variable	-	-	●	**Protocol C**	page 12
Urine	4.5 mL	variable	●	●	●	**Protocol D**	page 13
Buccal and nasal swabs	-	variable	●	●	●	**Protocol E**	page 14
Urogenital swabs	-	variable	●	●	●	**Protocol F**	page 15
Saliva	-	variable	●	●	●	**Protocol G**	page 16
Blood	up to 100 µL	96	●	-	-	**Protocol H**	page 17
		12	-	●	-	**Protocol I**	page 18
		variable	-	-	●	**Protocol J**	page 19

VIRAL DNA AND RNA PURIFICATION PROTOCOLS AND PIPETTING INSTRUCTIONS

Protocols A-C (pages 8-12) are recommended for viral DNA and RNA purification from human and animal plasma or serum samples. For viral DNA and RNA purification from urine, nasal and buccal swabs, saliva, urogenital swabs or blood follow **Protocols D-J*** (pages 13-19). ***Note.** In these protocols viral nucleic acids are isolated from collected cells, therefore cellular nucleic acids will also be present in the eluate.

Protocol A. Instructions for viral DNA and RNA purification from 200 µL plasma or serum using KingFisher Flex 96 and Microtiter deep well 96 plates

Before starting:

- Transfer the **Viral_NA_Flex** protocol file to the KingFisher Flex 96 as described on page 7.
- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.

Note. When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6.
- Organize proper handling of potentially infectious waste before beginning the procedure.
- Follow recommended safety instructions, as you will be working with potentially infectious material. Attention and care must be taken during the entire process.

1. Obtain four empty Thermo Scientific Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher Flex 96 KF plates.
2. Prepare the **Sample** plate (Microtiter deep well 96 plate) according to the table below.

Add the following reagents to the **Sample** plate and leave the plate at room temperature while the other plates are being filled.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Plasma, serum	200 µL
			Lysis Buffer (*supplemented with Carrier RNA*)	200 µL
			Proteinase K	50 µL

3. Prepare the other plates as follows:

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
2	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1	700 µL
3	Microtiter deep well 96 plate	Wash 2	Wash Buffer 2	800 µL
4	Microtiter deep well 96 plate	Wash 3	Wash Buffer 2	800 µL
5	KingFisher Flex 96 KF plate	Elution	Water, nuclease free	100 µL
6	KingFisher Flex 96 KF plate	Tip plate	-	-

4. Place a Thermo Scientific KingFisher Flex 96 tip comb for deep well magnets on a **Tip Plate** (an empty KingFisher Flex 96 KF plate).

5. Start the **Viral_NA_Flex** protocol with the KingFisher Flex 96 and load the plates according to the KingFisher display.
6. When the KingFisher Flex pauses (at the dispense step after the lysis step , approximately 15 minutes after starting the protocol run) add the **MagJET Magnetic Beads** suspension **resuspended well by vortexing** and isopropanol to the **Sample** plate.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Magnetic Beads*	25 µL
			100% isopropanol	450 µL

*Resuspend Magnetic Beads well by vortexing before use.

7. Place the **Sample** plate back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
8. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and turn off the instrument. The purified DNA or RNA is ready for use in downstream applications. Keep RNA or DNA samples on ice for immediate use, or store at -20°C or -70°C .

Protocol B. Instructions for viral DNA and RNA purification from 200 µL plasma or serum using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate

Before starting:

- Transfer the **Viral_NA_Duo** protocol file to the KingFisher Duo as described on page 7.
- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.
- Ensure you are using the KingFisher Duo 12-pin magnet head and heating block.

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6.
- Organize proper handling of potentially infectious waste before beginning the procedure.
- Follow recommended safety instructions, as you will be working with potentially infectious material. Attention and care must be taken during the entire process.

1. Obtain one empty Thermo Scientific Microtiter deep well 96 plate and one Thermo Scientific KingFisher Duo elution strip.
2. Prepare the **Viral NA plate** (Microtiter deep well 96 plate) according the table below.

Add the following reagents to the rows. Note that **row B** is reserved for the tip and should be left empty. Note that rows C, D and H are left empty.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Viral NA plate Microtiter deep well 96 plate	A	Sample	Plasma, serum sample	200 µL
			Lysis Buffer (*supplemented with Carrier RNA*)	200 µL
			Proteinase K	50 µL
	B	Tip	12-tip comb	-
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Wash 1	Wash Buffer 1	700 µL
	F	Wash 2	Wash Buffer 2	800 µL
	G	Wash 3	Wash Buffer 2	800 µL
	H	Empty	Empty	Empty

3. Fill the KingFisher Duo **Elution Strip** as follows. Make sure that the **Elution Strip** is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and **Water, nuclease free** for elution is pipetted into the correct wells.

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Water, nuclease free	100 µL

4. Place a Thermo Scientific KingFisher Duo 12-tip comb into **row B** on the **Viral NA plate**.
5. Start the **Viral_NA_Duo** protocol with the KingFisher Duo and load the plate and **Elution Strip.**

6. Switch on the KingFisher Duo instrument and ensure you are using the KingFisher Duo 12-pin magnet head and heating block. Start the **Viral_NA_Duo** protocol and load the plate and **Elution Strip** according to the KingFisher Duo display. Ensure that the **Elution Strip** is placed in the correct direction into the elution block and that the perforated end is facing towards the user. After all plates have been loaded the program will start.
7. When the KingFisher Duo pauses (at the dispense step after the lysis step, approximately 15 minutes after starting the protocol run) add the **MagJET Magnetic beads resuspended well by vortexing** and isopropanol to **row A** of the **Viral NA plate**.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Viral NA plate Microtiter deep well 96 plate	A	Sample	Magnetic beads	25 µL
			100% isopropanol	450 µL

8. Place the **Viral NA plate** back into the instrument and press **OK**. After the pause, the protocol will continue to the end.
9. When the protocol is completed, remove the plate and elution strip according to the instructions on the KingFisher Duo display and turn off the instrument. Transfer the eluate (which contains the purified DNA or RNA) to a new RNase-free tube and close immediately. The purified DNA or RNA is ready for use in downstream applications. Use the purified nucleic acids immediately or store at -20°C or -70°C . Keep DNA and RNA samples on ice after extraction and while working with it.

Protocol C. Instructions for manual viral DNA and RNA purification from 200 µL of plasma or serum

This protocol is based on transfer of liquids by pipetting through different purification steps rather than magnetic bead transfer as in KingFisher automatic protocols. It allows the kit to be used in various throughput applications using magnetic rack and manual or automated pipetting equipment. Protocols for different automated pipetting equipment should be optimized for each platform as well as the sample type used. To enable protocol optimization all buffers are available to purchase separately.

Before starting:

- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6.
- Organize proper handling of potentially infectious waste before beginning the procedure.
- Follow recommended safety instructions, as you will be working with potentially infectious material. Attention and care must be taken during the entire process.

1. Add 200 µL of **sample** (plasma, serum) to an empty 1.5-2.0 mL tube. Add 200 µL of **Lysis Buffer** (supplemented with Carrier RNA) and 50 µL of **Proteinase K**. Mix thoroughly by vortexing or pipetting. Incubate the sample for 15 min at 56°C in a thermomixer.
2. Add 25 µL **MagJET Magnetic Beads resuspended well by vortexing** and 450 µL of **isopropanol** (100%). Resuspend the magnetic beads by vortexing and briefly spin to remove droplets from inside of the lid. Place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2 minutes. Discard the supernatant by using a pipette.
3. Remove the magnetic rack and add 700 µL **Wash Buffer 1** (supplemented with isopropanol). Resuspend the magnetic beads by vortexing, place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2 minutes. Discard the supernatant by using a pipette.
4. Remove the magnetic rack and add 800 µL **Wash Buffer 2** (supplemented with ethanol). Resuspend the magnetic beads by vortexing, place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2 minutes. Discard the supernatant by using a pipette.
5. Repeat step 4 using 800 µL of **Wash Buffer 2**. Make sure that all the supernatant is removed completely (use a small volume pipette tip and make sure no droplets are left on the tube wall). Leave the tube on the magnet to dry at room temperature (15-25°C) for 5 minutes.
6. Remove the magnetic rack and add 100 µL **nuclease free water**. Resuspend the magnetic beads by vortexing and incubate for 5 minutes at 56°C in thermomixer. Place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2 minutes.
7. Carefully transfer supernatant containing viral DNA or RNA to a new, RNase free tube. Use the purified DNA or RNA immediately in downstream applications or store at -20°C or -70°C until use. Keep DNA and RNA samples on ice after extraction and while working with it.

Protocol D. Instructions for viral DNA and RNA purification from urine

Before starting:

- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.
- Prepare 0.5 M EDTA solution (pH 8.0).
- Prepare 0.9% NaCl solution or PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6.
- Transfer the **Viral_NA_Flex** protocol file to the KingFisher Flex 96 or **Viral_NA_Duo** protocol file to the KingFisher Duo instrument as described on page 7.

1	Add 0.5 mL of 0.5 M EDTA to 4.5 mL of urine (final concentration 50 mM). **Note:** Urine samples may contain insoluble salt precipitates that can reduce nucleic acid yields, thus limiting sample volume used for purification.
2	Centrifuge 10 min at 800 × g (~ 3,000 rpm). Discard the supernatant.
3	Resuspend the pellet in 200 µL of PBS or 0.9% NaCl.
4	For manual purification, proceed to **Step 1** of **Protocol C: Instructions for manual viral DNA and RNA purification from 200 µL of plasma or serum** on page 12. For automated purification using KingFisher Flex 96 Instrument proceed to **Step 1** of **Protocol A: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8. For automated purification using KingFisher Duo Instrument proceed to **Step 1** of **Protocol B: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate** on page 10.

Protocol E. Instructions for viral DNA and RNA purification from nasal and buccal swabs

Before starting:

- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.
- Prepare PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) or 0.9% NaCl solution.

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6.
- Transfer the **Viral_NA_Flex** protocol file to the KingFisher Flex 96 or **Viral_NA_Duo** protocol file to the KingFisher Duo instrument as described on page 7.

1	To collect a swab sample, scrape the swab 5-6 times against the inside of a cheek or nose.
2	Swirl the swab for 2-3 min in 200 µL of PBS or 0.9% NaCl.
3	For manual purification, proceed to **Step 1** of **Protocol C: Instructions for manual viral DNA and RNA purification from 200 µL of plasma or serum** on page 12. For automated purification using KingFisher Flex 96 Instrument proceed to **Step 1** of **Protocol A: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8. For automated purification using KingFisher Duo Instrument proceed to **Step 1** of **Protocol B: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate** on page 10.

Protocol F. Instructions for viral DNA and RNA purification from urogenital swabs

Before starting:

- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.
- Prepare TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) or 0.9% NaCl solution.

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6
- Transfer the **Viral_NA_Flex** protocol file to the KingFisher Flex 96 or **Viral_NA_Duo** protocol file to the KingFisher Duo instrument as described on page 7.

1A	**Cotton or brush tips:** Swab material collected using a shaft with cotton or brush tips should be placed into a long centrifuge tube (10-12 mL) containing 500 µL 0.9% NaCl or TE buffer, vortex gently, incubate on the shaking platform for 15-20 minutes and remove the shafts with tips from tubes. Collect cells by centrifugation at 5,000 × g for 10 minutes at 4-6˚C temperature. Discard the supernatant.
1B	**LBC medium:** If urogenital swabs were collected into LBC (liquid based cytology) medium, collect cells for DNA purification from 2 mL of medium by centrifugation at 5,000 × g for 10 minutes at 4-6˚C temperature. Discard the supernatant.
2	Resuspend the pellet from 1A or 1B in 200 µL of 0.9% NaCl or TE buffer.
3	For manual purification, proceed to **Step 1** of **Protocol C: Instructions for manual viral DNA and RNA purification from 200 µL of plasma or serum** on page 12. For automated purification using KingFisher Flex 96 Instrument proceed to **Step 1** of corresponding **Protocol A: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8. For automated purification using KingFisher Duo Instrument proceed to **Step 1** of **Protocol B: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate** on page 10.

Protocol G. Instructions for viral DNA and RNA purification from saliva

Before starting:

- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.
- Prepare TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) or PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4).

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described in page 5.
- Reconstitute the Carrier RNA as described on page 6.
- Transfer the **Viral_NA_Flex** protocol file to the KingFisher Flex 96 or **Viral_NA_Duo** protocol file to the KingFisher Duo instrument as described on page 7.

1	To collect cells centrifuge the saliva sample for 5 min at 3,000 × g. Discard the supernatant.
2	Resuspend cells in 200 µL of PBS or TE buffer.
3	For manual purification, proceed to **Step 1** of **Protocol C: Instructions for manual viral DNA and RNA purification from 200 µL of plasma or serum** on page 12. For automated purification using KingFisher Flex 96 Instrument proceed to **Step 1** of **Protocol A: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8. For automated purification using KingFisher Duo Instrument proceed to **Step 1** of **Protocol B: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate** on page 10.

Protocol H. Instructions for viral DNA and RNA purification from 50-100 µL of blood using KingFisher Flex 96 and Microtiter deep well 96 plates

Before starting:

- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.
- Transfer the **Viral_NA_Flex** protocol file to the KingFisher Flex 96 as described on page 7.

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6.

1. Obtain four empty Thermo Scientific Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher Flex 96 KF plates.
2. Prepare the **Sample** plate (Microtiter deep well 96 plate).

Note. Different amount of Lysis Buffer is added for the different starting volume of blood sample (final volume of blood with Lysis Buffer should be 400µL). Do not use more than 100 µL of blood.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well	
1	Microtiter deep well 96 plate	Sample	Whole blood	50 µL	100 µL
			Lysis Buffer (*supplemented with Carrier RNA*)	350 µL	300 µL
			Proteinase K	50 µL	

3. Proceed to **Step 3** of the **Protocol A: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Flex 96 and Microtiter deep well 96 plates** page 8 for automated viral DNA or RNA purification using KingFisher Flex 96.

Protocol I. Instructions for viral DNA and RNA purification from 50-100 µL of blood using KingFisher Duo with 12-pin magnet head and 96 deep well plate

Before starting:

- Transfer the **Viral_NA_Duo** protocol file to the KingFisher Duo as described on page 7
- Ensure you are using the KingFisher Duo 12-pin magnet head and heating block.
- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.
- Prepare TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) or PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4).

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6.

1. Obtain one empty Thermo Scientific Microtiter 96 deep well plate and one Thermo Scientific KingFisher Duo elution strip.
2. Prepare the **Viral NA plate** (Microtiter deep well 96 plate).

Add the following reagents to the rows. Note that **row B** is reserved for the tip and should be left empty. Note that rows C, D and H are left empty.

Note. Different amount of Lysis Buffer is added for the different starting volume of blood sample (final volume of blood with Lysis Buffer should be 400µL). Do not use more than 100 µL of blood.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well	
Viral NA plate Microtiter deep well 96 plate	A	Sample	Whole blood	50 µL	100 µL
			Lysis Buffer (*supplemented with Carrier RNA*)	350 µL	300 µL
			Proteinase K	50 µL	
	B	Tip	12-tip comb	-	
	C	Empty	Empty	Empty	
	D	Empty	Empty	Empty	
	E	Wash 1	Wash Buffer 1	700 µL	
	F	Wash 2	Wash Buffer 2	800 µL	
	G	Wash 3	Wash Buffer 2	800 µL	
	H	Empty	Empty	Empty	

3. Proceed to **Step 3** of the **Protocol B: Instructions for viral DNA and RNA purification from 200 µL of plasma or serum using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate** page 10 for for automated viral DNA or RNA purification using KingFisher Duo instrument.

Protocol J. Instructions for manual viral DNA and RNA purification from 50-100 µL of blood

Before starting:

- Supplement the required amount of **Lysis Buffer** with **Carrier RNA** (reconstituted). Add 25 µL of **reconstituted Carrier RNA** to each 1 mL of **Lysis Buffer** used and mix by pulse-vortexing or pipetting.
- Prepare TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) or PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4).

Note: When using the MagJET Viral DNA and RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1, Wash Buffer 2 as described on page 5.
- Reconstitute the dried Carrier RNA as described on page 6.

1. Add 50-100 µL of whole blood to an empty 1.5-2.0 mL tube. Add 350-300 µL of **Lysis Buffer** (supplemented with Carrier RNA) and 50 µL of **Proteinase K**. Mix thoroughly by vortexing or pipetting. Incubate the sample for 15 min at 56°C in a thermomixer.

Note. Different amount of Lysis Buffer is added for the different starting volume of blood sample (final volume of blood with Lysis Buffer should be 400µL). Do not use more than 100 µL of blood.

Proceed to Step 2 of the Protocol C: Instructions for manual viral DNA and RNA purification from 200 µL of plasma or serum on page 12 for manual viral DNA or RNA purification.

TROUBLESHOOTING

It is difficult to determine electrophoretically the yield of viral nucleic acids isolated from biological samples because of the Carrier RNA presented in the eluted samples. Therefore viral nucleic acids yield should be analysed by PCR (qPCR) or RT-PCR (RT-qPCR).

It is recommended to use the internal control, which is a convenient tool for the assessment and monitoring of extraction efficiency and PCR amplification respectively, for the quality of the purified nucleic acid, as well as for the exclusion of false negative results. Please refer to the user manual provided with the downstream detection assay for further directions on how to use an internal control.

Note. If viral nucleic acids are isolated from collected cells, cellular nucleic acids will also be present in the eluate. Please use the appropriate primers for viral nucleic acids detection.

Problem	Possible cause and solution
Low nucleic acid yield	**Incomplete resuspension of magnetic particles**. Resuspend the magnetic particles by vortexing before use. **Isopropanol was not added to the lysate.** Ensure that the isopropanol was added to the lysate during binding to MagJET Magnetic Beads step. **Isopropanol was not added to Wash Buffer 1.** Make sure that ethanol was added to Wash Buffer 1 prior to the first use as described on page 5 **Ethanol was not added to Wash Buffer 2:** Ensure that ethanol was added to Wash Buffer 2 as described on page 5. **Carrier RNA was not added to the lysate.** Reconstitute carrier RNA in Water nuclease free and mix with Lysis Solution as described on page 6. **Degraded carrier RNA.** Do not freeze-thaw the reconstituted Carrier RNA more than 10 times. Store at -20°C to -70°C.
Degraded RNA	**RNase contamination.** To avoid RNase contamination, wear gloves during all procedures and change gloves frequently. Use sterile, disposable RNase free pipette tips. Remove RNase contamination from non-disposable items and work surfaces. **Poor quality of samples.** Always use fresh samples or samples handled as recommended on page 6. For lysis, process the sample quickly to avoid degradation. **Purified RNA was not stored properly**. Purified RNA should be used immediately in downstream applications or stored at -20°C for later use. For prolonged storage at -70°C is recommended.
Carryover of the magnetic beads in the elution	Carryover of magnetic beads in the eluted DNA and RNA will not affect downstream applications. To remove the carryover MB from eluted RNA, simply magnetize the MB and carefully transfer to a new tube or plate.

SAFETY INFORMATION

Lysis Buffer for MagJET Viral Kit

Xn Harmful
Hazard-determining components of labelling: guanidinium thiocyanate
Risk phrases
R20/21/22: Harmful by inhalation, in contact with skin and if swallowed.
R32-52/53: Contact with acids liberates very toxic gas. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Safety phrases
23 Do not breathe gas/fumes/vapour/spray.
36/37/38 Wear suitable protective clothing, gloves, face/eye protection.
60 This material and its container must be disposed of as hazardous waste.
61 Avoid release to the environment. Refer special instructions/ safety data sheet

Proteinase K

Xn Harmful
Hazard-determining components of labelling: Proteinase, Tritirachium album serine
Risk phrases
42 May cause sensitisation by inhalation.
Safety phrases
23 Do not breathe gas/fumes/vapour/spray.
36 Wear suitable protective clothing.
45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
60 This material and its container must be disposed of as hazardous waste.

Wash Buffer 1 (conc.) for MagJET Viral Kit

Xn Harmful
Hazard-determining components of labelling: guanidinium chloride
Risk phrases
22 Harmful if swallowed.
36/38 Irritating to eyes and skin.
Safety phrases
23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37 Wear suitable protective clothing and gloves.
60 This material and its container must be disposed of as hazardous waste

PRODUCT USE LIMITATION
This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.
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