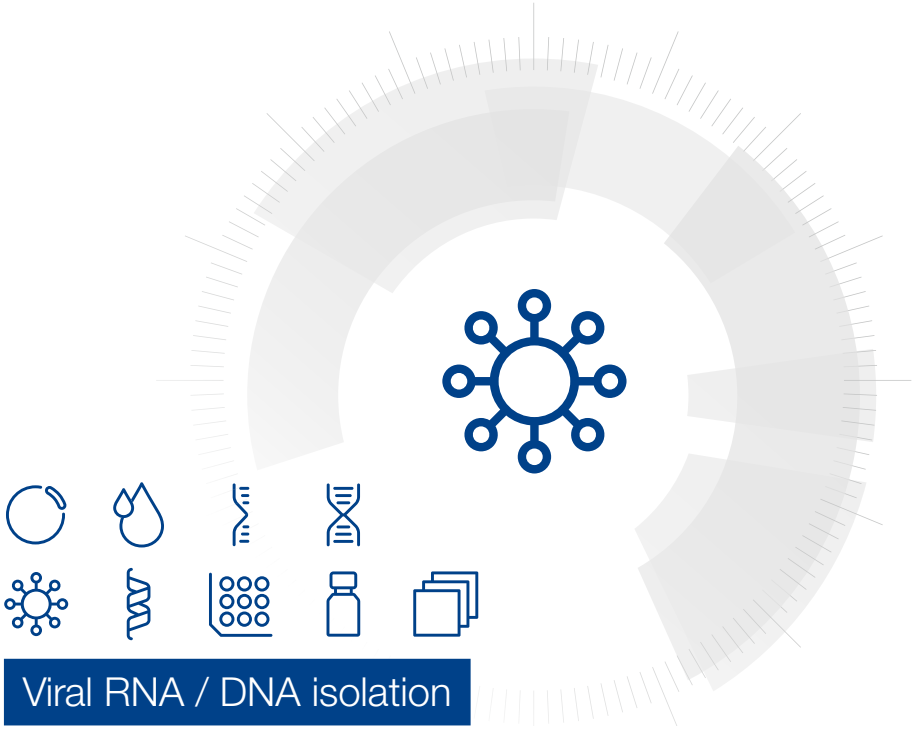
Bioanalysis

MACHEREY-NAGEL

User manual



Viral RNA / DNA isolation

■ NucleoMag® VET

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1 Components

1.1 Kit contents

	NucleoMag® VET		
REF	**1 × 96 preps 744200.1**	**4 × 96 preps 744200.4**	**100 × 96 preps 744200.100**
NucleoMag® B-Beads	2 × 1.25 mL	10 mL	22 × 10 mL
Lysis Buffer VL1	30 mL	100 mL	4 × 500 mL
Binding Buffer VEB	110 mL	3 × 110 mL	7 × 1000 mL
Wash Buffer VEW1	75 mL	300 mL	7 × 1000 mL
Wash Buffer VEW2	75 mL	300 mL	7 × 1000 mL
Elution Buffer VEL	30 mL	125 mL	3 × 500 mL
Carrier RNA*	400 µg	4 × 400 µg	85 × 400 µg
Carrier RNA Buffer	500 µL	4 × 500 µL	3 × 15 mL
Proteinase K (lyophilized)*	75 mg	3 × 75 mg	65 × 75 mg
Proteinase Buffer PB	8 mL	15 mL	18 × 15 mL
Leaflet	1	1	1

* For preparation of working solutions and storage conditions see section 3.

1.2 Material to be supplied by user

Product	REF	Pack of
Separation plate for magnetic beads separation, e.g., Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24
Lysis tubes for incubation of samples and lysis, e.g., Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Elution plate for collecting purified nucleic acids, e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24
e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 300 µL flat-bottom wells)	740673	20
For use of kit on KingFisher™ Flex instrument: 96-well Accessory Kit A for KingFisher™ (Square-well Blocks, Deep-well Tip Combs, Elution Plates for 4 × 96 NucleoMag® VET preps using KingFisher™ Flex platform)	744950	1 set

Reagents:

- 80 % ethanol

1.3 About this user manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at www.mn-net.com.

Please contact Technical Service regarding information about changes of the current user manual compared to previous or updated revisions.

2 Product description

2.1 The basic principle

The **NucleoMag® VET** kit is designed for the isolation of viral DNA or RNA and bacterial DNA from cell-free body fluids such as serum or plasma, swab, blood or homogenized tissue sample suspensions. This kit provides reagents and magnetic beads for isolation of 96 samples from 100 – 200 µL. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with a Lysis Buffer VL1 containing chaotropic ions supported by Proteinase K digestion. For binding of nucleic acids to the paramagnetic beads, Binding Buffer VEB and the NucleoMag® B-Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers VEW1 and VEW2 and 80 % ethanol. Residual ethanol from previous wash steps is removed by airdrying. Finally, highly pure viral RNA/DNA is eluted with low salt Elution Buffer VEL or water. Purified viral RNA/DNA can directly be used for downstream applications. The **NucleoMag® VET** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

NucleoMag® VET is designed for rapid manual and automated small-scale preparation of viral RNA/DNA from cell-free body fluids such as serum or plasma samples, blood samples, homogenized tissue suspensions or sample materials as described in chapter 5. The kit is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information) or other magnetic separation systems (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The purified RNA/DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

NucleoMag® VET allows easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag® SEP on the automation platform.

The NucleoMag® VET kit is intended for use by professional users such as technicians and physicians experienced and trained in molecular biological techniques including experience with swabs and other potentially infectious, veterinary sample materials. The product is intended for research use only.

2.3 Magnetic separation systems

For use of **NucleoMag® VET**, the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information, section 6.2). The kit can also be used with other common separators.

Magnetic separator	Separation plate or tube
NucleoMag® SEP (MN REF 744900)	Square-well Block (MN REF 740481)
Tecan Te-MagS™	1.5 mL tubes without lid (Sarstedt)

Static magnetic pins

Separators with static magnetic pins, for example, NucleoMag® SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

Movable magnetic systems

Separators with moving magnetic pins: Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.4 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and wash steps:

- Load 600 µL dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing step.

Adjusting shaker speed for the elution step:

- Load 100 µL dyed water to the wells of the collection plate and proceed as described above.

2.5 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads/binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended using the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension efficiency	Speed	Number of tips needed
Magnetic mix	+	++	Low
Shaker	++	++	Low
Pipetting	+++	+*	High

2.6 Elution procedures

Purified viral RNA/DNA can be eluted directly with the supplied Elution Buffer VEL. Elution can be carried out in a volume of ≥ 50 µL. It is essential to cover the NucleoMag® Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, high elution volumes might be necessary to cover the whole pellet.

* 8-channel pipetting device

3 Storage conditions and preparation of working solutions

Attention:

VL1, VEB, VEW1, VEW2, Carrier RNA Buffer, the NucleoProtect® VET Blood and Swab tubes and the NucleoProtect® VET reagent contain chaotropic salt (e.g. guanidine hydrochloride, guanidinium thiocyanate and/or sodium perchlorate) which can form highly reactive compounds when combined with bleach (sodium hypochlorite)! DO NOT add bleach or acidic solutions directly to the sample preparation waste. Wear suitable protective clothing, gloves and safety goggles!

- All components of the **NucleoMag® VET** kit should be stored at 15 – 25 °C and are stable until: see package label.
- All buffers are delivered ready to use.

Buffer VIA, necessary to process NucleoProtect® VET-stabilized blood samples, may form precipitates during storage. Check Buffer VIA for precipitates and incubate at 25 – 30 °C for approx. 30 minutes to redissolve any precipitated material. Gently shake the bottle until a homogenous solution has formed.

Before starting any **NucleoMag® VET** protocol, prepare the following:

- **Proteinase K:** Before first use of the kit, add 3.35 mL Proteinase Buffer PB to each vial of the **lyophilized Proteinase K**. Dissolved Proteinase K solution should be stored in aliquots at - 20 °C.
- **Carrier RNA:** Before first use of the kit, add 500 µL Carrier RNA Buffer to each vial **lyophilized Carrier RNA**. Store dissolved Carrier RNA solution in aliquots at - 20 °C.

Note: Due to the production procedure and the small amount of Carrier RNA contained in the vial, the Carrier RNA may hardy be visible.

NucleoMag®VET			
REF	**1 × 96 preps 744200.1**	**4 × 96 preps 744200.4**	**100 × 96 preps 744200.100**
Proteinase K (lyophilized)	1 vial (75 mg) Add 3.35 mL Proteinase Buffer	3 vials (75 mg/vial) Add 3.35 mL Proteinase Buffer to each vial	65 vials (75 mg/vial) Add 3.35 mL Proteinase Buffer to each vial
Carrier RNA (lyophilized)	1 vial (400 µg) Add 500 µL Carrier RNA Buffer	4 vials (400 µg/vial) Add 500 µL Carrier RNA Buffer to each vial	85 vials (400 µg/vial) Add 500 µL Carrier RNA Buffer to each vial

4 Safety instructions

When working with the **NucleoMag® VET** kit, wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at http://www.mn-net.com/msds).

Caution: Guanidine hydrochloride in Lysis Buffer VL1, sodium perchlorate in buffer VEB, VEW1, VEW2 and guanidinium thiocyanate in Carrier RNA Buffer, the **NucleoProtect® VET Blood and Swab tubes** and the **NucleoProtect® VET reagent** can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The inactivation capacity of the **NucleoProtect VET** reagent has been demonstrated for various background matrices and viruses known to have different envelope structures as well as susceptibilities to disinfectants: FMDV (small non-enveloped virus), BTV-5 (large non-enveloped virus), LSDV (large enveloped virus), PPRV and BCoV (enveloped virus). Claims for inactivation of other viruses or background matrices cannot be made. Samples must be incubated for at least 30 min for complete inactivation.

The waste generated with the **NucleoMag® VET** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according to local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Preparation of sample materials

a) Blood and serum / plasma samples

A sample volume of 100 – 200 µL blood can be used. Do not use higher volumes. When using less than 200 µL samples, adjust with PBS buffer to 200 µL.

b) NucleoProtect® VET-stabilized blood samples

Vortex the NucleoProtect® VET Blood tube or tube containing a blood sample stabilized in NucleoProtect® VET reagent thoroughly for 30 seconds. Briefly centrifuge the tube for 10 seconds at 200 x *g* to remove drops form inside the lid. Transfer 200 µL stabilized blood sample. Optional: Recap the NucleoProtect® VET Blood tube with a secondary sealing cap, see ordering information .

c) Tissue samples

Homogenize tissue samples. Typically 5 – 10 mg sample material can be homogenized in 400 µL PBS buffer using a bead based homogenizer. If necessary, higher amounts of sample material can be used (up to 25 mg). It should be considered that the copurified total nucleic acid may cause inhibition in the subsequent PCR assays. After homogenization of the tissue, centrifuge and use up to 200 µL clear supernatant for the purification protocol. If using less than 200 µL, adjust with PBS buffer to a final volume of 200 µL.

For isolation of viral RNA:
Tissue samples can be also disrupted in a buffer containing chaotropic salt (e.g., Buffer RA1, see ordering information) and beta-mercaptoethanol or TCEP reducing agent (see ordering information).

d) Swab samples / NucleoProtect® VET-stabilized swab samples

Incubate the swabs with PBS, sodium chloride, or cell culture medium for 30 min with shaking. Remove and squeeze out the swab. Proceed with 200 µL of the particle-free buffer or medium for purification protocol.

Vortex the NucleoProtect® VET Swab tube or tube containing a swab sample stabilized in NucleoProtect® VET reagent thoroughly for 30 seconds . Briefly centrifuge the tube for 10 seconds at 200 x *g* to remove drops form inside the lid. Transfer 200 µL stabilized swab sample.

e) Feces

Mix 1 volume of feces (e.g., 500 µL) with an equal volume of PBS buffer. Mix vigorously by vortexing for 1 min. Allow the particles to settle down or centrifuge with low speed (e.g., at 500 x *g*). For difficult-to-lyse bacteria, mechanical disruption (e.g., treatment using suitable glass beads) may be required. Take the supernatant and use 200 µL for the purification protocol.

f) TRIzol® lysis

For sample materials such as semen, a TRIzol® lysis may be required. Homogenize 10 – 30 mg tissue or up to 250 µL blood with 1 mL TRIzol® reagent to manufacturer's instructions. After phase separation by centrifugation, remove aqueous, colorless (upper) phase (approximately 400 µL). For further processing, start with step 2 of the purification protocol by mixing 400 µL of the aqueous phase with 600 µL Buffer VEB and 20 µL NucleoMag® B-Beads.

g) Milk samples

Usually a sample volume higher than 200 µL is used. Typically, 1 mL of a normal milk sample is centrifuged (e.g., 11.000 x *g* for 3 min). Discard the supernatant and resuspend the pellet in 400 µL PBS. Proceed with 200 µL sample input for the purification protocol.

Sour milk samples need an additional pretreatment step. Therefore, incubate the sour milk particles/chunk in an appropiate amount of lysis buffer for 1 – 3 h at 56 °C (ideally shaking). Pellet residual particles and proceed with 400 µL of the lysate with the step 2 of the purification protocol.

6 Protocols for manual use or use on liquid handling systems

6.1 Isolation of viral RNA / DNA and bacterial DNA from blood, tissue homogenates, serum, plasma, other body fluids, swabs, (NucleoProtect® VET-)stabilized swab samples and washes

Preparation of sample material

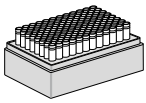
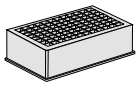
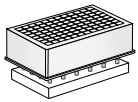
The standard protocol is related to a volume of 200 µL (homogenized) sample. For the preparation of different sample materials (e.g., tissue, swabs, feces), please see the indications at section 5.

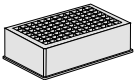
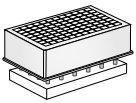
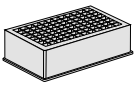
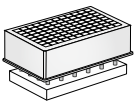
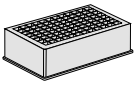
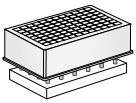
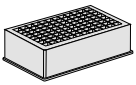
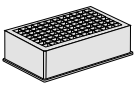
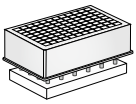
Protocol at a glance

- For hardware requirements refer to section 2.3.
- For detailed information on each step see page 16.

Before starting the preparation:

- Check that Proteinase K and Carrier RNA were prepared according to section 3.

1	Lyse sample	**200 µL (homogenized) sample** **20 µL Proteinase K** **4 µL Carrier RNA** **180 µL VL1** **Mix** **RT, 15 min**	
2	Bind nucleic acid to NucleoMag® B-Beads	**20 µL B-Beads** **600 µL VEB**	
		Mix by shaking for 5 – 10 min at RT (Optional: Mix by pipetting up and down)	
		Remove supernatant after 2 min separation	

3	Wash with VEW1	**Remove Square-well Block from NucleoMag® SEP** **600 µL VEW1**	
		Resuspend: Shake 1 min at RT	
		Remove supernatant after 2 min separation	
4	Wash with VEW2	**Remove Square-well Block from NucleoMag® SEP** **600 µL VEW2**	
		Resuspend: Shake 1 min at RT	
		Remove supernatant after 2 min separation	
5	Wash with 80 % ethanol	**Remove Square-well Block from NucleoMag® SEP** **600 µL 80 % ethanol**	
		Resuspend: Shake 1 min at RT	
		Remove supernatant after 2 min separation	
6	Air-dry magnetic beads	**Air-dry 10 min at RT**	
7	Elute RNA / DNA	**Remove Square-well Block from NucleoMag® SEP** **50-100 µL VEL**	
		Shake 5 min at RT (Optional: Mix by pipetting up and down)	
		Separate 2 min and transfer RNA / DNA into elution plate / tubes	

Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers. It is recommended using a Square-well Block for separation (see ordering information, section 6.2). Alternatively, isolation of RNA/DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

1 Lyse sample

Pre-dispense **20 µL Proteinase K** and **200 µL of sample** to a suitable reaction tube. Add **180 µL Buffer VL1** to the reaction tube. Optional: add **4 µL** of **the Carrier RNA** stock solution to the reaction tube. Mix well by repeated pipetting up and down and incubate at **room temperature** for **15 min** with shaking. Alternatively, lysis step can be performed in Tube Strips (see ordering information).

Following the lysis incubation, spin down to collect any sample from the lysis tube lids and transfer each lysate to the wells of a Square-well Block.

2 Bind nucleic acid to magnetic beads

Add **20 µL resuspended B-Beads** and **600 µL Buffer VEB** to the lysed sample.

Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

NucleoMag® B-Beads and Buffer VEB can be premixed.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP a magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Do not disturb the attracted beads while aspirating the supernatant.

3 Wash with VEW1

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **600 µL Buffer VEW1** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

4 Wash with VEW2

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **600 µL Buffer VEW2** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

5 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **600 µL 80 % ethanol** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

6 Air-dry magnetic beads

Air-dry the magnetic bead pellet for **10 min** at **room temperature**.

7 Elute RNA / DNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add desired volume of **Buffer VEL (50 – 100 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5 min** at **room temperature**. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5 min** at **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified nucleic acids to either elution plates or tube strips (see ordering information).

6.2 Isolation of viral RNA / DNA from NucleoProtect® VET -stabilized blood samples

Protocol for the isolation of viral RNA and DNA from NucleoProtect® VET Blood tubes or blood samples stabilized in NucleoProtect® VET reagent.

For preparation of stabilized blood samples see section 5.

Note: Isolation of nucleic acids from NucleoProtect® VET-stabilized and non-stabilized blood samples can be performed in parallel. The alteration of the protocol for stabilized blood sample is limited to sample lysis and adjustment of binding conditions only.

Protocol at a glance

- For hardware requirements refer to section 2.3.
- For detailed information on each step see page 16.

Before starting the preparation:

For the processing of NucleoProtect® VET-stabilized blood samples, additional buffer VIA is needed (REF 744206, see ordering information). Check Buffer VIA for precipitates, see section 3.

1	**Lyse sample**	200 µL stabilized blood sample 350 µL VIA 20 µL Proteinase K Mix RT, 15 min
2	**Bind nucleic acid to NucleoMag® B-Beads**	20 µL B-Beads 450 µL VEB
		Mix by shaking for 5 – 10 min at RT *(Optional: Mix by pipetting up and down)*
		Remove supernatant after 2 min separation
3	**Wash with VEW1**	Proceed with step 3 of the standard protocol of section 6.1

7 Protocols for magnetic rod systems

These protocols are designed for the use of the NucleoMag® VET kit on magnetic rod systems (e.g. KingFisher™ systems, MagnetaPure 32 or other magnetic rod based instruments).

- For detailed information on each step see page 16.

Please contact our Technical Support Bioanalysis (tech-bio@mn-net.com) for method files or more detailed information on specific automation platforms.

7.1 General setup for magnetic rod systems

This overview serves as a guideline for the general setup of plates, columns, or reaction vessels of the respective instrument for the NucleoMag® VET kit.

Depending on the instrument, lysis of sample material can either be performed on the instrument or external.

Positions can represent different formats that are defined by the protocol and instrument (e.g. complete plates (96-well format devices), single rows (12-well format), single columns (8-well format) or individual wells; cartridge based systems).

External lysis - lysis is not performed on the instrument itself

(for sample materials that require advanced handling or clarification via centrifugation)

Position	Step	Buffer	Volume
1	Lysis/Binding	Lysate*	404 µL
		B-Beads	20 µL
		VEB	575 µL
2	Wash 1	VEW1	600 µL
3	Wash 2	VEW2	600 µL
4	Wash 3	80 % Ethanol	600 µL
5	Elution	VEL	100 µL

* Includes 200 µL sample, 180 µL Buffer VL1, 20 µL Proteinase K and 4 µL Carrier RNA

On-deck lysis - lysis is performed on the instrument; for liquid or fully homogenized sample materials

Position	Step	Buffer	Volume
1	Lysis	Sample	200 µL
		Proteinase K	20 µL
		VL1	180 µL
		Carrier RNA	4 µL
	Binding	B-Beads	20 µL
	[add after lysis]	VEB	575 µL
2	Wash 1	VEW1	600 µL
3	Wash 2	VEW2	600 µL
4	Wash 3	80 % Ethanol	600 µL
5	Elution	VEL	100 µL

7.2 Setup for the isolation of viral RNA / DNA from NucleoProtect® VET-stabilized blood samples

Isolation of viral RNA and DNA from NucleoProtect® VET Blood tubes or blood samples stabilized in NucleoProtect® VET reagent.

Note: Isolation of nucleic acids from stabilized blood samples and other sample material can be performed within the same instrument run. The alteration of the protocol for stabilized blood sample is limited to sample lysis and adjustment of binding conditions only.

For preparation of stabilized blood samples see section 5.

Before starting the preparation:

- Check that Proteinase K was prepared according to section 3.

For the processing of NucleoProtect® VET-stabilized blood samples, additional buffer VIA is needed (REF 744206, see ordering information 9.2). Check Buffer VIA for precipitates, see section 3.

On-deck lysis - lysis is performed on the instrument; for liquid or fully homogenized sample materials

Position	Step	Buffer	Volume
1	Lysis	Sample	200 µL
		VIA	330 µL
		Proteinase K	20 µL
	Binding	B-Beads	20 µL
	[add after lysis]	VEB	425 µL
2	Wash 1	VEW1	600 µL
3	Wash 2	VEW2	600 µL
4	Wash 3	80 % Ethanol	600 µL
5	Elution	VEL	100 µL

7.3 Detailed protocol for KingFisher™ Flex

Note: The required method file 'NucleoMag® _VET_lyse_Flex' or other method files for the instrument is available at Technical Support Bioanalysis (tech-bio@mn-net.com).

Important: Always prepare the deep-well block with samples first and add reagents exactly in the order as given below.

Before starting the preparation:

- Check that Proteinase K and Carrier RNA were prepared according to section 3.
- 96-well Accessory Kit A for KingFisher™ (see ordering information)

1	**Prepare sample / lysis plate (part I)** Dispense **20 µL Proteinase K solution** to each well of the 96-well deep-well block. Add **200 µL blood sample / homogenized tissue sample** to each well of the 96-well deep-well block, mix by pipetting up and down. Add **180 µL Buffer VL1** and mix by pipetting up and down 3 times. Optional: Shake at 1,000 rpm for 15 min at room temperature. Continue with the preparation of the wash and elution plates before adding magnetic beads and binding buffer to the sample plate.
2	**Prepare wash and elution plates** Wash plates: Fill **600 µL Buffer VEW1** to each well of an empty Thermo 96-well deep well plate. Fill **600 µL Buffer VEW2** to each well of an empty Thermo 96-well deep well plate. Fill **600 µL 80 % ethanol** to each well of an empty Thermo 96-well deep well plate. Elution plate: Fill **100 µL Buffer VEL** to each well of an empty Thermo 200 µL 96-well plate.
3	Prepare sample / lysis plate (part II) Add **20 µL B-Beads** and **575 µL buffer VEB** to each well of the sample / lysis plate.

4 Run purification protocol on instrument

Start the isolation of nucleic acids on the KingFisher™ Flex instrument.

Start the method file 'NucleoMag® VET'.

Insert plates as indicated on the KingFisher™ instrument display.

Method starts with a mixing step (combined lysis and binding step) after setting up the last plate to the instrument.

5 Remove eluted nucleic acids

The instrument stops after the final elution step. Follow the instructions on the instrument's display and unload the plates from the instrument.

Purified RNA / DNA can be used for further PCR based analysis.

8 Support protocols

8.1 Isolation of viral RNA (PRRS) from porcine sperm samples

Before starting the preparation:

- The additional Lysis Buffer RA1 is required (see ordering information 9.2).

1	**Precipitation** Centrifuge 1 mL sperm sample for 4 min at 12.000 x *g*. Discard the supernatant after centrifugation.
2	**Lyse sample** Add 400 Lysis Buffer RA1 and mix by pipetting. Incubate for 10 min at 70° C.
3	**Clear lysate** Centrifuge the lysed sample for 1 min. at 15.000 x *g* Use 400 µL of the cleared lysate and proceed with step 2 of standard protocol, see section 6.2.

9 Appendix

9.1 Troubleshooting

Problem	Possible cause and suggestions
Poor yield / low sensitivity	*Insufficient elution buffer volume* • Beads pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step* • Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation too short or aspiration speed too high.
Low purity / low sensitivity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.
Poor performance of nucleic acids in downstream applications	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the 80 % ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications. *Ethanol evaporation from wash buffers* • Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.

Problem	Possible cause and suggestions
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

9.2 Ordering information

Product	REF	Pack of
NucleoMag® VET	744200.1 744200.4 744200.100	1 × 96 preps 4 × 96 preps 100 × 96 preps
NucleoProtect® VET stabilization and inactivation reagent	740750.50 740750.500	50 mL 500 mL
NucleoProtect® VET Blood Tubes	740755	50
Secondary sealing caps for NucleoProtect® VET Blood Tubes	740756	100
Buffer VIA	744206	150 mL
NucleoMag® SEP	744900	1
Square-well Blocks	740481 740481.24	4 24
Self adhering PE Foil	740676	50 sheets
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Elution Plate U-bottom	740486.24	24
96-well Accessory Kit A for KingFisher™ (set consists of Square-well Blocks, Deep-well Tip Combs, set consists of Elution Plates for 4 × 96 **NucleoMag® VET** preps using KingFisher™ Flex platform)	744950	1 set
Buffer RA1 (60 mL)	740961	60 mL
Reducing Agent TCEP	740395.107	107 mg
NucleoSpin® VET	740842.10 740842.50 740842.250	1 x 10 preps 1 x 50 preps 1 x 250 preps

Visit ***www.mn-net.com*** for more detailed product information.

9.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY NAGEL literature. No other statements or representations, written or oral, by MACHEREY NAGELS employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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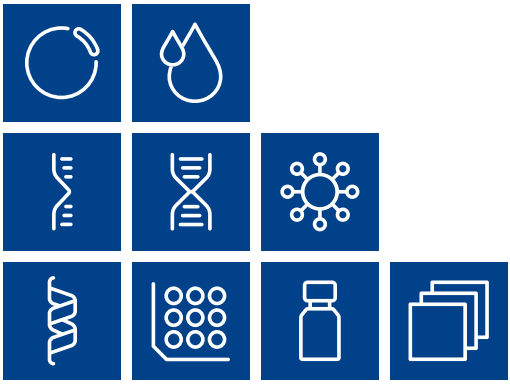
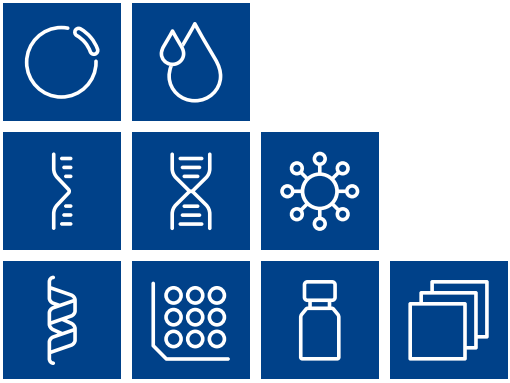


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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