Viral RNA / DNA isolation

User manual

NucleoMag® VET

April 2013 / Rev. 01

MACHEREY-NAGEL
www.mn-net.com

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1 Components

1.1 Kit contents

	NucleoMag® VET	
	1x 96 preps	**4 x 96 preps**
REF	**744200.1**	**744200.4**
NucleoMag® B-Beads	2.5 mL	10 mL
Lysis Buffer VL	2 x 12.5 mL	100 mL
Binding Buffer VEB	110 mL	3 x 110 mL
Wash Buffer VEW1	75 mL	4 x 75 mL
Wash Buffer VEW2	75 mL	4 x 75 mL
Elution Buffer VEL	30 mL	125 mL
Carrier RNA*	400 µg	4 x 400 µg
Carrier RNA Buffer	500 µL	4 x 500 µL
Proteinase K (lyophilized)*	75 mg	3 x 75 mg
Proteinase Buffer PB	5 mL	15 mL
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* 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) e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 300 µL flat-bottom wells)	740486.24 740673	24 20
For use of kit on KingFisher® 96 instrument: e.g., KingFisher® 96 Accessory Kit A (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® 96 Virus preps using KingFisher® 96 platform)	744950	1 set

Reagents:

- 80 % ethanol

2 Product description

2.1 The basic principle

The **NucleoMag® VET** kit is designed for the isolation of viral DNA or RNA from cell-free body fluids such as serum or plasma, 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 VL 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 or homogenized tissue suspensions. 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.

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). 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:

Buffers VL, VEB, VEW1, and VEW2 contain chaotropic salt! Wear gloves and goggles!

- All components of the **NucleoMag® VET** kit should be stored at room temperature (18–25 °C) and are stable for up to one year.
- All buffers are delivered ready-to-use.

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.

	NucleoMag® VET	
	1 x 96 preps	**4 x 96 preps**
REF	**744200.1**	**744200.4**
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
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

4 Safety instructions

The following components of the **NucleoMag® VET** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

4.1 Risk and safety phrases

Component *Inhalt*	Hazard contents *Gefahrstoff*	Hazard symbol *Gefahrstoff-symbol*	Risk phrases *R-Sätze*	Safety phrases *S-Sätze*
VL	Guanidine hydrochloride 50–66 % *Guanidinhydrochlorid 50–66 %*	Xn	R 22-36/38	S 26-37/39
VEB	Sodium perchlorate 20–40 % + ethanol 35–55 % *Natriumperchlorat 20–40 % + Ethanol 35–55 %*	Xn*	R 10-22	S 13-16
VEW1, VEW2	Sodium perchlorate 5–20 % + ethanol 20–35 % *Natriumperchlorat 5–20 % + Ethanol 20–35 %*	*	R 10	S 16
Carrier RNA Buffer	Guanidinium thiocyanate 30–60 % *Guanidiniumthiocyanat 30–60 %*	Xn* N*	R 20/21/22-32-52/53	S 13-61
Proteinase K	Proteinase K, lyophilized *Proteinase K, lyophilisiert*	Xn	R 36/37/38-42	S 22-24-26-36/37

Risk phrases

R 10	Flammable. *Entzündlich.*
R 22	Harmful by inhalation. *Gesundheitsschädlich beim Verschlucken.*
R 20/21/22	Harmful by inhalation, in contact with skin, and if swallowed. *Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut.*
R 32	Contact with acids liberates very toxic gas. *Entwickelt bei Berührung mit Säure sehr giftige Gase.*
R 36/38	Irritating to eyes and skin. *Reizt die Augen und die Haut.*
R 36/37/38	Irritating to eyes, respiratory system, and skin. *Reizt die Augen, Atmungsorgane und die Haut.*

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

** Hazard labeling not necessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

Risk phrases

R 42	May cause sensitization by inhalation. *Sensibilisierung durch Einatmen möglich.*
R 42/43	May cause sensitization by inhalation and skin contact *Sensibilisierung durch Einatmen und Hautkontakt möglich.*
R 52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. *Schädlich für Wasserorganismen, kann in Gewässern längerfristig schädliche Wirkungen haben.*

Safety phrases

S 13	Keep away from food, drink, and animal feedstuffs. *Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.*
S 16	Keep away from sources of ignition – No Smoking! *Von Zündquellen fernhalten – Nicht rauchen.*
S 22	Do not breathe dust. *Staub nicht einatmen.*
S 24	Avoid contact with the skin. *Berührung mit der Haut vermeiden.*
S 26	In case of contact with the eyes, rinse with plenty of water and seek medical advice. *Bei Berührung mit den Augen gründlich mit Wasser abspülen und Arzt konsultieren.*
S 36/37	Wear suitable protective clothing and gloves. *Bei der Arbeit geeignete Schutzhandschuhe und Schutzkleidung tragen.*
S 37/39	Wear suitable gloves and eye / face protection. *Bei der Arbeit geeignete Schutzhandschuhe und Schutzbrille / Gesichtsschutz tragen.*
S 61	Avoid release to the environment. Refer to special instructions / safety data sheet. *Freisetzung in die Umwelt vermeiden. Besondere Anweisungen einholen / Sicherheitsdatenblatt zu Rate ziehen.*

4.2 GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
VL	Guanidine hydrochloride 50–66 % *Guanidinhydrochlorid 50–66 %*	 Warning *Achtung*	302, 315, 319	280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313
VEB	Sodium perchlorate 20–40 % + ethanol 35–55 % *Natriumperchlorat 20–40 % + Ethanol 35–55 %*	 Danger *Gefahr*	226, 302	210, 233, 301+312, 330, 403+235
VEW1, VEW2	Sodium perchlorate 5–20 % + ethanol 20–35 % *Natriumperchlorat 5–20 % + Ethanol 20–35 %*	 Warning *Achtung*	226	210, 233, 403+235
Carrier RNA Buffer	Guanidinium thiocyanate 30–60 % *Guanidiniumthiocyanat 30–60 %*	 Warning *Achtung*	302, 412, EU031	260, 273, 301+312, 330
Proteinase K	Proteinase K, lyophilized *Proteinase K, lyophilisiert*	 Danger *Gefahr*	315, 317, 319, 334, 335	261, 280, 302+352, 304+340, 305+351+338, 312, 333+313, 337+313, 342+311, 363, 403+233

Hazard phrases

H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 315	Causes skin irritation. *Verursacht Hautreizungen.*
H 317	May cause an allergic skin reaction. *Kann allergische Hautreaktionen verursachen.*
H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. *Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*
H 335	May cause respiratory irritation. *Kann die Atemwege reizen.*

Hazard phrases

H 412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
EUH 031	Contact with acids liberates toxic gas. *Entwickelt bei Berührung mit Säure giftige Gase.*

Precaution phrases

P 210	Keep away from heat/sparks/open flames/hot surfaces – No smoking. *Von Hitze / Funken / offener Flamme / heißen Oberflächen fernhalten. Nicht rauchen*
P 233	Keep container tightly closed. *Behälter dicht verschlossen halten.*
P 260	Do not breathe dust / fume / gas / mist / vapours / spray. *Dampf nicht einatmen.*
P 261	Avoid breathing dust. *Einatmen von Staub vermeiden.*
P 273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P 280	Wear protective gloves / eye protection. *Schutzhandschuhe / Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER or doctor /physician if you feel unwell. *Bei Verschlucken: Bei Unwohlsein Giftinformationszentrum oder Arzt anrufen.*
P 302+352	IF ON SKIN: Wash with plenty of soap and water. *Bei Kontakt mit der Haut: Mit viel Wasser und Seife waschen.*
P 304+341	IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing. *Bei Einatmen: Bei Atembeschwerden an die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
P 305+351+313	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing *BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.*
P 330	Rinse mouth. *Mund ausspülen.*
P 333+313	If skin irritation or a rash occurs: Get medical advice / attention. *Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician. *Bei Symptomen der Atemwege: Giftinformationszentrum oder Arzt anrufen.*
P 337+313	Get medical advice / attention. *Bei anhaltender Augenreizung: Ärztliche Rat einholen / ärztliche Hilfe hinzuziehen.*
P 363	Wash contaminated clothing before reuse. *Kontaminierte Kleidung vor erneutem Tragen waschen.*
P 403+235	Store in a well ventilated place. Keep cool. *Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.*

For further information please see Material Safety Data Sheets ***(www.mn-net.com)***.
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ***(www.mn-net.com)****.*

⚠ The symbol shown on labels refers to the precaution phrases of this section.
Das auf Etiketten dargestellte Symbol weist auf die P-Sätzen dieses Kapitels hin.

5 Protocols

5.1 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) 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 ammounts 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).

c) Swab samples

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

d) 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 or treatment using suitable glas beads may be required. Take the supernatant and use 200 µL for the purification protocol.

e) 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.

5.2 Isolation of viral RNA/DNA and bacterial DNA from blood, tissue homogenates, serum, plasma, other body fluids and washes

Preparation of sample material

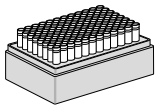
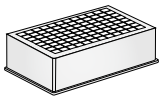
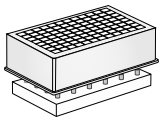
The standard protocol is related to a volume of 200 µL (homogenized) sample. For the preparation of different sample materials (e.g., tissue, swabs, feces), please see the indications at section 5.1.

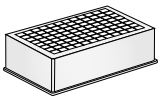
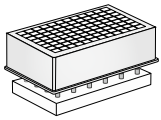
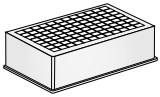
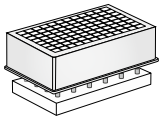
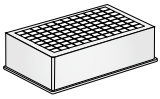
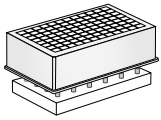
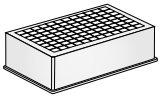
Protocol-at-a-glance

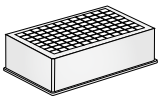
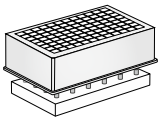
- For hardware requirements refer to section 2.3.
- For detailed information on each step see page 18.

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 VL** **Mix** **RT, 15 min**	
2	Bind nucleic acid to NucleoMag® B-Beads	**600 µL VEB** **20 µL B-Beads**	
		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 viral 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). 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 VL** 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 pre-mixed.

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 viral RNA/DNA to either elution plates or tube strips (see ordering information).

5.3 Detailed protocol for KingFisher® Flex 96

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

Important: Always prepare 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.
- KingFisher® Accessory Kit A (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 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 VL** and mix by pipetting up and down 3 times.

Optional: Shake at 1000 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 **600 µL Buffer VEB** to each well of the sample / lysis plate.

4 Run purification protocol on instrument

Start the isolation of viral RNA / DNA on the KingFisher® Flex 96 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 viral RNA / DNA

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

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

6 Appendix

6.1 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 RNA 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.

Poor performance of RNA in downstream applications *(continued)*	*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.
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.

6.2 Ordering information

Product	REF	Pack of
NucleoMag® VET	744200.1 744200.4	1 x 96 preps 4 x 96 preps
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
KingFisher® 96 Accessory Kit A (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® VET preps using KingFisher® 96 platform)	744950	1 set
Buffer RA1 (50 mL)	740961	50 mL
Reducing Agent TCEP	740395.107	107 mg

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

6.3 Product use restriction/warranty

NucleoMag® VET kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN VITRO*-diagnostic use. Please pay attention to the package of the product. *IN VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-270
tech-bio@mn-net.com