Bioanalysis

MACHEREY-NAGEL

# User manual

## Viral RNA and DNA and bacterial DNA

■ NucleoSpin® VET

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# Contact MN


## Germany and international

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-0
Toll-free: 0800 26 16 000 (Germany only)
E-mail: info@mn-net.com

## Technical Support Bioanalysis

Tel.: +49 24 21 969-333
E-mail: support@mn-net.com

## USA

MACHEREY-NAGEL Inc.
924 Marcon Blvd. · Suite 102 · Allentown PA, 18109 · USA
Toll-free: 888 321 6224 (MACH)
E-mail: sales-us@mn-net.com

## France

MACHEREY-NAGEL SAS
1, rue Gutenberg – BP135 · 67720 Hoerdt Cedex · France
Tel.: +33 388 68 22 68
E-mail: sales-fr@mn-net.com

MACHEREY-NAGEL SAS (Société par Actions Simplifiée) au capital de 186600 €
Siret 379 859 531 00020 · RCS Strasbourg B379859531 · N° intracommunautaire FR04 379 859 531

## Switzerland

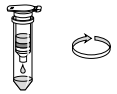
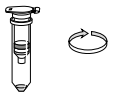
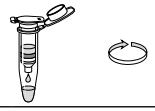
MACHEREY-NAGEL AG
Hirsackerstr. 7 · 4702 Oensingen · Switzerland
Tel.: +41 62 388 55 00
E-mail: sales-ch@mn-net.com

*www.mn-net.com*

# NucleoSpin® VET - Viral and bacterial DNA and viral RNA isolation from veterinary samples

## Protocol at a glance (Rev. 02)

The protocol at a glance serves as a general overview only. We strongly recommend to carefully read the detailed protocol section of the product's user manual before first use.
*Note: The FastTrack protocol for processing plasma, serum and swab wash solutions, and the protocols for nucleic acid extraction from NucleoProtect VET-stabilized blood and swab samples are only described in the detailed user manual.*

| | 5.1<br>**200 µL serum or plasma** | 5.2<br>**100 µL animal blood** | 5.3<br>**5 – 10 mg tissue** | 5.4<br>**dry swab** | 5.5<br>**wet swab** | 5.6<br>**feces** |
|---|---|---|---|---|---|---|
| 1 **Sample lysis** | 10 µL Liquid PK<br>200 µL sample<br>200 µL SVL<br>mix 10 min 1,400 rpm<br>+2.5 µL Carrier RNA<br>mix<br>incubate at RT 3 min<br>Quick spin | 10 µL Liquid PK<br>100 µL blood<br>100 µL PBS<br>200 µL SVL<br>mix 10 min 1,400 rpm<br>+2.5 µL Carrier RNA<br>mix<br>incubate at RT 3 min | 5 – 10 mg tissue in Bead Tube<br>400 µL PBS<br>agitate<br>remove beads<br>transfer 200 µL supernatant<br>+10 µL Liquid PK<br>+200 µL SVL<br>mix 10 min at 1,400 rpm | 400 µL PBS<br>insert dry swab<br>30 min at RT shaking<br>remove swab<br>1 min 2,000 x g<br>transfer 200 µL supernatant<br>+10 µL Liquid PK<br>+200 µL SVL<br>mix 10 min at 1,400 rpm<br>+2.5 µL Carrier RNA<br>mix<br>3 min RT | remove swab from medium<br>transfer 300 – 1000 µL medium<br>1 min 2,000 x g<br>transfer 200 µL supernatant<br>+10 µL Liquid PK<br>+200 µL SVL<br>mix 10 min 1,400 rpm<br>+2.5 µL Carrier RNA<br>mix<br>3 min RT | mix feces with tenfold PBS<br>1 min 2,000 x g<br>transfer 250 µL<br>+250 µL SVL<br>mix<br>+10 µL Liquid PK<br>mix 10 min 1,400 rpm<br>opt. : +2.5 µL Carrier RNA<br>mix<br>3 min RT<br>3 min 15,000 x g<br>recover and continue with 400 µL supernatant |
| 2 **Adjust binding conditions** | +200 µL ethanol<br>mix<br>RT, 5 min<br>Quick spin | | | | | |
| 3 **Bind viral RNA / DNA** | Load sample<br>(~ 610 µL)<br>4,000 x g, 3 min | | | | | |
| 4 **Wash silica membrane** |  **1st wash** 400 µL SVW1 11,000 x *g*, **30 s**<br>**2nd wash** 400 µL SVW2 11,000 x *g*, **30 s**<br>**3rd wash** 200 µL SVW2 20,000 x *g*, 5 min | | | | | |
| 5 **Dry silica membrane** |  56 °C, 5 min with open lid | | | | | |
| 6 **Elute RNA / DNA** |  100 µL RNase-free $H_2O$ (70 °C)<br>RT, 3 min<br>20,000 x g, 3 min | | | | | |

**MACHEREY-NAGEL GmbH & Co. KG** · Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-270 · tech-bio@mn-net.com · ***www.mn-net.com***

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# 1 Components

## 1.1 Kit contents

| | NucleoSpin® VET | | |
|---|---|---|---|
| **REF** | **10 preps 740842.10** | **50 preps 740842.50** | **250 preps 740842.250** |
| Lysis Buffer SVL | 13 mL | 25 mL | 125 mL |
| Wash Buffer SVW1 | 6 mL | 30 mL | 125 mL |
| Wash Buffer SVW2 (Concentrate)* | 6 mL | 12 mL | 50 mL |
| RNase-free $H_2O$ | 13 mL | 13 mL | 13 mL |
| Carrier RNA (lyophilized) | 300 µg | 300 µg | 300 µg |
| Liquid Proteinase K | 120 µL | 600 µL | 2 × 1.5 mL |
| NucleoSpin® VET Columns (light red rings, plus Collection Tubes) | 10 | 50 | 250 |
| Collection Tubes (2 mL) | 30 | 150 | 750 |
| Collection Tubes (1.5 mL) for lysis and elution | 20 | 100 | 500 |

## 1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol (to prepare Wash Buffer SVW2 and to adjust RNA / DNA binding conditions); non-denaturated ethanol is recommended
- PBS (Phosphate Buffered Saline) for the processing of blood, tissue, swabs, and feces

Consumables

- Disposable pipette tips (aerosol barrier pipette tips are recommended to avoid cross-contamination)

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

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Heating block: 56 °C for column drying and 70 °C for elution buffer (not required for fast track protocol)
- Personal protection equipment (e.g., lab coat, gloves, goggles)
- MN Bead Tubes Type D (see ordering information) for the processing of tissue material

## 1.3 About this user manual

It is strongly recommended to read the detailed protocol sections of this user manual if using the **NucleoSpin® VET** kit for the first time. However, experienced users may refer to the Protocol at a glance. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at ***www.mn-net.com***.

# 2 Product description

## 2.1 The basic principle

The **NucleoSpin® VET** kit provides protocols for several different veterinary sample types (serum, plasma, cell-free biological fluids, milk, blood, tissue, swabs, feces). The protocols differ in their initial pretreatment and lysis of the sample and comprised viruses in order to cope with the different properties of samples. Sample and virus lysis is achieved by Lysis Buffer SVL, which is a highly concentrated solution of chaotropic ions, and digestion with Proteinase K, which is supplied in the kit. After lysis, all protocols follow the same procedure. The mixture is applied to the NucleoSpin® VET Column and nucleic acids bind. Contaminations (potential PCR inhibitors) like salts, metabolites, and soluble macromolecular cellular components are removed in simple wash steps with alcoholic buffers SVW1 and SVW2. The nucleic acids are eluted in RNase-free water and are ready-for-use in all common downstream applications.

Carrier RNA improves binding and recovery of low-concentrated viral nucleic acids and is used in some – but not all -protocols.

## 2.2 Kit specifications

**NucleoSpin® VET** kit is designed for the rapid preparation of highly pure nucleic acids (ss/dsRNA viruses, ss/dsDNA viruses, bacterial DNA) from veterinary specimens, such as plasma, serum, cell-free biological fluids, milk, blood, tissue samples, dry and wet swabs, and feces.

- Reduced risk of cross-contamination due to closed systems. All listed sample types can be processed with the same **standard protocol** procedure after indicated sample pretreatment.
- The **NucleoSpin® VET** kit is suited to process per preparation 200 µL plasma / serum, 100 µL blood, 5 – 10 mg tissue, one dry or wet swab, or approx. 100 mg feces.
- The prepared nucleic acids are suitable for applications, such as automated fluorescent DNA sequencing, RT-PCR, PCR, or any kind of enzymatic reaction.
- The detection limit of viruses depends on the individual detection procedures, such as, in-house nested (RT-)PCR or qRT-PCR. We highly recommend using internal standards as well as positive and negative controls to monitor the purification, amplification, and detection processes.
- **Carrier RNA** (poly(A)-RNA: poly(A)-potassium salt, prepared from ADP with polynucleotide phosphorylase) is included for optimal performance.
- **Liquid Proteinase K** is included to facilitate adequate lysis of protein in the samples.

Processing is carried out by means of centrifugation. For Research Use Only.

| Table 1: Kit specifications at a glance | |
|---|---|
| Parameter | NucleoSpin® VET |
| Technology | Silica membrane technology |
| Format | Mini spin columns |
| Sample material | 200 µL serum, plasma, cell-free biological fluids, milk, 100 µL blood, 5 – 10 mg tissue, one dry or wet swab, or approx. 100 mg feces |
| Fragment size | approx. 100 bp – 50 kb |
| Elution volume | 100 µL |
| Preparation time | 20 – 40 min |

## 2.3 Remarks regarding sample quality and preparation

Different kinds of veterinary samples can be processed with **NucleoSpin® VET** kit. For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and non-viscous sample before loading onto the **NucleoSpin® VET** Columns. Therefore, check all samples (especially old or frozen ones) for precipitates. Avoid clearing plasma / serum samples by centrifugation / filtration before the SVL-lysis step, because viruses may be associated with particles or aggregates.

## 2.4 Remarks regarding elution

- Pure nucleic acids are finally eluted under low ionic strength conditions with RNase-free $H_2O$.
- Elution can be performed in a single step with water as indicated in the protocol, obtaining at least 80 % of the bound nucleic acids. To improve sensitivity, this eluate can be used in a second elution step increasing the efficiency of elution and concentration of viral nucleic acids slightly. Alternatively, a second elution step can be performed with an additional volume of water releasing practically all bound nucleic acids but resulting in a lower concentrated, combined eluate.
- A high RNA / DNA concentration in the elution fraction is of highest importance and desirable for all typical downstream applications. This is of particular interest if the total volume of a reaction mixture is limited as this in turn limits the possible amount of added DNA / RNA. Due to a high default elution volume, classical RNA / DNA purification kits often result in weakly concentrated RNA / DNA, if only small samples are processed. Such RNA / DNA often even requires a subsequent concentration before it can be used for typical downstream applications.

## 2.5 Remarks regarding quality control

In accordance with MACHEREY-NAGEL's Quality Management System, each component of **NucleoSpin® VET** kits is tested against predetermined specifications to ensure consistent product quality.

# 3 Storage conditions and preparation of working solutions

*Attention: Buffers SVL and SVW1 contain guanidine salts! Wear gloves and goggles!*

- Check all components for damages after receiving the kit. If kit contents like buffer bottles or blister packages are damaged, contact MACHEREY-NAGEL. Do not use damaged kit components.
- Upon arrival, the **NucleoSpin® VET** kit should be stored at room temperature (15 – 25 °C) and is stable until: see package lable. It is NOT required to open the kit on delivery and remove individual components for separate storage.
- After first time use, it is recommended to store Liquid Proteinase K at 4 °C or -20 °C.
- Use RNase-free equipment.

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

- **Carrier RNA** (300 µg) is delivered in lyophilized form. Dissolve Carrier RNA in RNase-free water to obtain a working solution (100 ng/µL) see table below. Store Carrier RNA working solution at -20 °C. Due to the production procedure and the small amount of Carrier RNA contained in the vial, the Carrier RNA may hardly be visible in the vial.
- **Wash Buffer SVW2**: Add the indicated volume (see on the bottle or table below) of ethanol (96 – 100 %; non-denatured ethanol is recommended) to **Wash Buffer SVW2 Concentrate**. Mark the label of the bottle to indicate that the ethanol is added. Store **Wash Buffer SVW2** at room temperature.
- **Liquid Proteinase K** is ready to use. After first time use, store liquid Proteinase K at 4 °C or -20 °C.

| | NucleoSpin® VET | | |
|---|---|---|---|
| **REF** | **10 preps**<br>**740842.10** | **50 preps**<br>**740842.50** | **250 preps**<br>**740842.250** |
| Wash Buffer SVW2 (Concentrate) | 6 mL<br>Add 24 mL ethanol | 12 mL<br>Add 48 mL ethanol to each bottle | 50 mL<br>Add 200 mL ethanol |
| Carrier RNA | 300 µg<br>Add 3 mL RNase-free $H_2O$ | 300 µg<br>Add 3 mL RNase-free $H_2O$ | 300 µg<br>Add 3 mL RNase-free $H_2O$ |

# 4 Safety instructions

When working with the **NucleoSpin® 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 www.mn-net.com/msds).

Caution:

Buffer SVL, Buffer SVW1, the **NucleoProtect® VET** Blood and Swab tubes and the NucleoProtect® VET reagent contain chaotropic salt (e.g. guanidine hydrochloride and/or guanidinium thiocyanate) 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.

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). Samples must be incubated for at least 30 min for complete inactivation. Claims for inactivation of other viruses or background matrices cannot be made.

The waste generated with the **NucleoSpin® 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 Protocols for viral RNA and DNA purification

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 instructions in sections 5.2 – 5.6.

After lysis, all protocols follow the same procedure (step 2, which is the adjustment of binding conditions with 200 µL EtOH).

## 5.1 Standard protocol for serum, plasma, milk, or cell-free biological fluids

**Before starting the preparation:**

- For frozen plasma and serum samples we recommend not to freeze-thaw more than once before processing.
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature.
- Preheat RNase-free $H_2O$ (for elution) to 70 °C.

| | | |
|---|---|---|
| **1** | **Lyse sample** | |
| | Provide **10 µL Liquid Proteinase K** in a Collection Tube (1.5 mL, provided). | **10 µL Proteinase K** |
| | *Proteinase K may be pipetted onto the side of the inner tube wall. Visually inspect, that the Liquid Proteinase K has been actually deposited into the tube!* | |
| | Add **200 µL sample** to the tube and mix moderately. | **+200 µL sample** |
| | Add **200 µL Lysis Buffer SVL** to the tube. | **+200 µL SVL** |
| | **Mix** the tube for **10 min at 1,400 rpm** (e.g. on an Eppendorf Thermoshaker) at room temperature. | **Mix, 10 min 1,400 rpm** |
| | If necessary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |
| | Add **2.5 µL Carrier RNA working solution** (100 ng/µL) to the tube. | **+2.5 µL Carrier RNA** |
| | Mix the tube content by vortexing or pipetting up and down. | **Mix** |
| | Incubate for **3 min** at **room temperature**. | **RT, 3 min** |
| | If neccesary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |

| | | |
|---|---|---|
| 2 | **Adjust binding conditions**<br><br>Add **200 µL ethanol** (96 – 100 %) to the tube and mix by vortexing (10 – 15 s).<br><br>Incubate for **5 min** at **room temperature**.<br><br>Briefly centrifuge the Collection Tube (**~ 1 s** at **~ 2,000 x *g***) to remove drops from the lid (short spin only).<br><br>Do not centrifuge at a higher *g*-force in this step! | **+200 µL EtOH**<br><br>**RT, 5 min**<br><br>**~ 1 s,**<br>**~ 2,000 x *g*** |
| 3 | **Bind viral RNA / DNA**<br><br>**Load the lysate (610 µL)** onto a **NucleoSpin® VET Column** and centrifuge **3 min** at **4,000 x *g***.<br><br>*If the lysate is not completely drawn through the membrane, repeat the centrifugation at higher g-forces (15,000 – 20,800 x g for 1 min). If the lysate still does not completely pass the membrane, check the amount of sample material used.*<br><br>Place the **NucleoSpin® VET Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step. | **Load sample**<br>**3 min,**<br>**4,000 x *g*** |

**4 Wash and dry silica membrane**

**1 st wash** — **+400 µL SVW1**

Add **400 µL Wash Buffer SVW1** to the NucleoSpin® VET Column.

Centrifuge **30 s** at **11,000 x *g***. — **30 s, 11,000 x *g***

Place the **NucleoSpin® VET Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

**2nd wash** — **+400 µL SVW2**

Add **400 µL Wash Buffer SVW2** to the NucleoSpin® VET Column.

Centrifuge **30 s** at **11,000 x *g***. — **30 s, 11,000 x *g***

Place the **NucleoSpin® VET Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

*Note: Make sure that residual buffer from the previous step is washed away with Buffer SVW2, especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim, flush it with Buffer SVW2.*

**3rd wash** — **+200 µL SVW2**

Add **200 µL Wash Buffer SVW2** to the NucleoSpin® VET Column.

Centrifuge **for 5 min** at **20,000 x *g*** (or full speed). — **5 min, 20,000 x *g***

Place the **NucleoSpin® VET Column** in a clean Elution Tube (1.5 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

Incubate the assembly for **5 min** at **56 °C** with open column lid. — **56 °C, 5 min**

**5 Elute RNA / DNA**

Add **100 µL RNase-free $H_2O$** (preheated to 70 °C) onto the column. — **+100 µL RNase-free $H_2O$ (70 °C)**

*Note: A smaller volume than the recommended 100 µL or an unheated elution buffer can also be used, but this may significantly reduce elution efficiency.*

Incubate for **3 min** at **room temperature**. — **RT, 3 min**

Centrifuge **3 min** at **20,000 x *g*** to elute nucleic acid from the column. — **3 min, 20,000 x *g***

Keep eluted RNA / DNA on ice or freeze for storage.

## 5.2 Pretreatment of animal blood

**Before starting the preparation:**

For animal blood, collection in common blood collection tubes containing anticoagulant (e.g. EDTA) is recommended. Blood treated with EDTA, citrate, or heparin as anticoagulant can be used as sample material. Blood samples can be either fresh or frozen. Freeze-thawing more than once can lead to impaired sample quality. We recommend using 100 µL of blood from species containing non-nucleated erythrocytes. However, highly elevated cell counts due to inflammatory or neoplastic diseases may strongly increase the host nucleic acid content of a sample. In this case, we recommend to reduce the initial sample input to 50 µL blood. For blood samples containing nucleated erythrocytes (e.g. from fish, birds, reptiles), use less than 50 µL blood.

- Check that PBS is available.
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature.
- Preheat RNase-free $H_2O$ (for elution) to 70 °C.

| | | |
|---|---|---|
| **1** | **Sample lysis** | |
| | Provide **10 µL Liquid Proteinase K** in a Collection Tube (1.5 mL, provided). | **10 µL Liquid Proteinase K** |
| | *Proteinase K may be pipetted onto the side of the inner tube wall. Visually inspect, that the Liquid Proteinase K has been actually deposited into the tube!* | |
| | Add **100 µL animal blood** and **100 µL PBS** into the tube. If less than 100 µL blood is used, increase the PBS volume to reach a total volume of 200 µL blood-PBS mix. | **+100 µL blood +100 µL PBS** |
| | Add **200 µL Lysis Buffer SVL** to the tube. | **+200 µL SVL** |
| | Mix the tube for 10 min at 1,400 rpm (e.g. on an Eppendorf Thermoshaker) at room temperature. | **Mix 10 min 1,400 rpm** |
| | If necessary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~1 s ~2,000 x *g*** |
| | Add **2.5 µL Carrier RNA working solution** (100 ng/µL) to the tube. | **+2,5 µL Carrier RNA** |
| | **Mix** the tube content by vortexing or pipetting up and down. | **Mix** |
| | Incubate for **3 min** at **room temperature**. | **RT, 3 min** |
| | If neccesary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |

After sample pretreatment, continue with the standard protocol procedure in section 5.1 step 2 (page 11):
*Adjust binding conditions* (which is the addition of 200 µL ethanol).

## 5.3 Pretreatment of tissue samples

**Before starting the preparation:**

- *Note: It should be considered that high amounts of co-purified nucleic acid (DNA/RNA of host cells) may cause inhibition of DNA polymerase in the subsequent PCR assays. Should this be the case, we recommend to lower sample input volume.*
- *Note: Addition of carrier RNA is not recommended for tissue samples because tissue samples typically already contain considerable amounts of DNA.*
- *Note: Different tissue types can vary widely with regard to texture and rigidity, cell types, and content of host nucleic acids and inhibitory substances. Further, the localization of pathogen nucleic acids in the tissue may vary depending on tissue type, pathogen, and stage of infection. Therefore, suitability of the pretreatment protocols in this handbook should be evaluated for each new combination of tissue and pathogen. For e.g. spleen samples, which contain high amounts of host nucleic acids, the use of less than 5 – 10 mg is recommended.*
- Check that PBS is available.
- Check that MN Bead Tubes Type D are available.
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature.
- Preheat RNase-free $H_2O$ (for elution) to 70 °C.

**1 Lyse tissue and viruses**

Transfer **5 – 10 mg tissue sample** (e.g. poultry liver) to a **MN Bead Tube Type D** (see ordering information).

**Transfer 5 – 10 mg tissue in Bead Tube**

Add **400 µL PBS** to the tube and close it.

**+400 µL PBS**

Agitate the tube in a mixer-mill in order to homogenize the sample. For a Retsch mixer-mill a homogenization duration of approximately 30 seconds at 30 Hertz is recommended. Other homogenization devices might require different settings.

**agitate**

**Remove** the **beads** from the tube (e.g. using a magnet to attach the beads to the lid while opening the tube).

**remove beads**

Centrifuge the tube for **1 min** at **2,000 x *g*** in order to remove tissue debris.

**1 min, 2,000 x *g***

*Note: Do not increase centrifugation time or force in order to avoid sedimentation of viral particles.*

| | |
|---|---|
| Transfer **200 µL of the cleared supernatant** into a new reaction tube. | **Transfer 200 µL supernatant +10 µL Proteinase K** |
| Add **10 µL Liquid Proteinase K** to the tube and mix moderately.<br>*Proteinase K may be pipetted onto the side of the inner tube wall. Visually inspect, that the Liquid Proteinase K has been actually deposited into the tube!* | |
| Add **200 µL Lysis Buffer SVL** to the tube. | **+200 µL SVL** |
| **Mix** the tube for **10 min** at **1,400 rpm** (e.g. on an Eppendorf Thermoshaker) at room temperature. | **Mix 10 min 1,400 rpm** |
| If necessary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |
| *Note: Addition of Carrier RNA is not recommended due to the high content of nucleic acid in tissue samples.* | |

Continue with section 5.1 step 2 (page 11): *Adjust binding conditions* (which is the addition of 200 µL ethanol).

## 5.4 Pretreatment of dry swab samples

**Before starting the preparation:**

- Different kinds of dry swabs can be used to collect veterinary samples (e.g. poultry anal swabs).
- Check if PBS is available.
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature.
- Preheat RNase-free $H_2O$ (for elution) to 70 °C.

| | | |
|---|---|---|
| 1 | **Sample lysis** | |
| | Add **400 µL PBS** (Phosphate Buffered Saline) into a 2 mL tube (not provided). | **400 µL PBS** |
| | *Note: For swabs absorbing more than approximately 200 µL solution, an increase of the PBS volume is recommended to recover a total volume of 200 µL PBS-sample solution after the dry swab was incubated in and with-drawn from the PBS.* | |
| | After taking up sample material with a dry swab (e.g. poultry anal swab) **insert the swab** into the tube containing the PBS. | **Insert dry swab** |
| | **Incubate at room temperature** while shaking the tube for 30 minutes. | **incubate at RT for 30 min** |
| | **Remove the swab** from the tube. | **Remove swab** |
| | Centrifuge the tube for **1 min at 2,000 x *g*** in order to remove sample debris. | **1 min 2,000 xg** |
| | *Note: Do not increase centrifugation time or force in order to avoid sedimentation of viral particles.* | |
| | **Transfer 200 µL** of the supernatant into a fresh tube. | **Transfer 200 µL supernatant** |
| | Add **10 µL Liquid Proteinase K** into the tube and mix moderately. | **+10 µL Proteinase K** |
| | *Proteinase K may be pipetted onto the side of the inner tube wall. Visually inspect, that the Liquid Proteinase K has been actually deposited into the tube!* | |
| | Add **200 µL Lysis Buffer SVL** to the tube. | **+200 µL SVL** |
| | **Mix** the tube for 10 min at 1,400 rpm (e.g. on an Eppendorf Thermoshaker) at room temperature. | **Mix, 10 min 1,400 rpm** |

| | |
|---|---|
| If necessary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |
| **Add 2.5 µL Carrier RNA** working solution (100 ng/µL) to the tube. | **+2.5 µL Carrier RNA** |
| **Mix** the tube content by vortexing or pipetting up and down. | |
| Incubate for **3 min** at **room temperature**. | **Mix RT, 3 min** |
| If neccesary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |

Continue with section 5.1 step 2 (page 11): *Adjust binding conditions* (which is the addition of 200 µL ethanol).

## 5.5 Pretreatment of wet swabs (swabs within virus transport medium, VTM)

**Before starting the preparation:**

- *Note: Transport or stabilization media may vary in chemical composition depending on the manufacturer. Compatibility with the NucleoSpin® VET kit must be tested or validated before use.*
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature.
- Preheat RNase-free $H_2O$ (for elution) to 70 °C.

**1 Sample lysis**

| | |
|---|---|
| **Remove the swab** from the tube transport medium (e.g. Sigma-Virocult®). | **Remove swab from medium** |
| **Transfer** approximately **300 – 1000 µL of transport medium** into a fresh tube (not provided). | **Transfer 300 – 1000 µL medium** |
| Centrifuge the tube for **1 min at 2,000 x *g*** in order to remove sample debris. | **1 min 2,000 x *g*** |
| *Note: Do not increase centrifugation time or force in order to avoid sedimentation of viral particles.* | |
| **Transfer 200 µL** of the supernatant into a fresh tube. | **Transfer 200 µL** |
| Add **10 µL Liquid Proteinase K** into the tube and mix moderately. | **+10 µL Proteinase K** |
| *Proteinase K may be pipetted onto the side of the inner tube wall. Visually inspect, that the Liquid Proteinase K has been actually deposited into the tube!* | |
| Add **200 µL Lysis Buffer SVL** to the tube. | **+200 µL SVL** |
| **Mix** the tube for **10 min at 1,400 rpm** (e.g. on an Eppendorf Thermoshaker) at room temperature. | **Mix, 10 min 1,400 rpm** |
| If necessary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |
| **Add 2.5 µL Carrier RNA** working solution (100 ng/µL) to the tube. | **+2.5 µL Carrier RNA** |
| **Mix** the tube content by vortexing or pipetting up and down. | |
| Incubate for **3 min** at **room temperature**. | **Mix RT, 3 min** |
| If neccesary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |

Continue with section 5.1 step 2 (page 11): *Adjust binding conditions* (which is the addition of 200 µL ethanol).

## 5.6 Pretreatment of feces

**Before starting the preparation:**

- *Note: Different fecal types can vary widely with regard to texture, rigidity, water content, content of host and pathogen nucleic acids, and inhibitory substances. Therefore, suitability of the pretreatment protocols in this handbook should be evaluated for each new combination of fecal sample type and pathogen. Hard and/or dry feces might require some mechanical support for disruption and/or a higher volume of PBS in order to obtain a liquid slurry.*
- *Note: Feces can vary greatly in consistency and inhibitor content. The processing of samples with very high inhibitor contents may therefore require a reduction in the sample volume if necessary. For difficult samples, it is therefore recommended to reduce the amount of feces to less than 100 mg in 1 mL of PBS.*
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature.
- Preheat RNase-free $H_2O$ (for elution) to 70 °C.

| | | |
|---|---|---|
| **1** | **Sample lysis** | |
| | **Mix** a **feces** sample with a **tenfold (10x)** amount of **PBS** (either per weight or volume), e.g. mix 100 mg or 100 µL feces with 1 mL PBS and mix thoroughly in order to resuspend particular matter and obtain a fecal suspension. | **Mix feces with tenfold PBS** |
| | Centrifuge the tube for **1 min** at **2,000 x *g*** in order to remove debris. | **1 min 2,000xg** |
| | *Note: Do not increase centrifugation time or centrifugation speedin order to avoid sedimentation of resuspended viral particles.* | |
| | **Transfer 250 µL** from the supernatant into a fresh tube. | **Transfer 250 µL** |
| | *Note: Floating particles should not be transfered but discarded together with the sediment.* | |
| | **Add 250 µL** of Lysis Buffer **SVL** into the tube to the supernatant. | **+250 µL SVL** |
| | **Mix** thoroughly for 30 seconds by vortexing. | **Mix** |
| | If neccesary, briefly centrifuge the Collection Tube (**~ 1 s at ~ 2,000 x *g***) to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |
| | **Add 10 µL** of **Liquid Proteinase K**. | **+10 µL Liquid Proteinase K** |
| | **Mix** the tube for **10 min at 1,400 rpm** (e.g. on an Eppendorf Thermoshaker) at room temperature. | **Mix, 10 min 1,400 rpm** |
| | Optional: **Add 2,5 µL Carrier-RNA** solution (100 ng/µL) into the tube and **mix** the tube content by vortexing or pipetting up and down. | **opt. : +2.5 µL Carrier RNA** |
| | Incubate for **3 min** at **room temperature**. | **Mix**<br>**RT, 3 min** |
| | Centrifuge the tube for **3 min** at approximately **15,000 x *g*** in order to remove unlysed material. | **~ 3 min, ~ 15,000 x *g*** |
| | **Recover 400 µL** of the **supernatant** and transfer it into a fresh tube. | **Recover and continue with 400 µL supernatant** |
| | *Note: This step removes unlysed material; do only use supernatant and discard sediment!* | |

Continue with section 5.1 step 2 (page 11): *Adjust binding conditions* (which is the addition of 200 µL ethanol).

## 5.7 Pretreatment of NucleoProtect® VET-stabilized swab samples

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

**Before starting the preparation:**

- For the processing of NucleoProtect® VET-stabilized swab samples, additional buffer PFN is needed (REF 740121.5, see ordering information).
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature (18 – 25 °C).
- Preheat RNase-free $H_2O$ (for elution) to 70 °C.

| | | |
|---|---|---|
| **1** | **Sample lysis** | |
| | Vortex the NucleoProtect® VET Swab tube or the tube containing a swab sample stabilized in NucleoProtect® VET reagent thoroughly for 30 seconds. Briefly centrifuge the tube for 10 sec. at 200 x *g* to remove drops form inside the lid. | |
| | Withdraw **200 µL of the solution** and transfer it into a fresh tube (e.g. 1.5 mL). | **transfer 200 µL solution into fresh tube** |
| | Add **100 µL Lysis Buffer SVL, 100 µL water, 2 µL Buffer PFN** and vortex for 10 – 15 s. | **+100 µL SVL**<br>**+100 µL water**<br>**+2 µL PFN** |
| | *Note: Buffer SVL, water and PFN can be premixed in a Mastermix. For 10 samples, mix 1000 µL SVL, 1000 µL water and 20 µL PFN.* | |
| | Add **10 µL of Liquid Proteinase K** into the tube and mix moderately. | **+10 µL Liquid Proteinase K** |
| | Mix the tube for **10 min** at **1,400 rpm** (e.g. on an Eppendorf Thermoshaker) at room temperature. | |
| | Add **2.5 µL Carrier-RNA working solution** (100 ng/µL) to the tube. | **opt.: +2.5 µL Carrier RNA** |
| | Mix the tube content by vortexing or pipetting up and down. | **Mix** |
| | Incubate for 3 min at room temperature. | **RT, 3 min** |
| | If necessary, briefly centrifuge the tube (~ 1 s at ~ 2.000 xg) to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |
| **2** | After sample pretreatment, continue with the standard protocol procedure in section 5.1 step 2 (page 11): Adjust binding conditions (which is the addition of 200 µL ethanol). | |

## 5.8 Pretreatment of NucleoProtect® VET-stabilized blood samples

*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 pretreatment only.*

**Before starting the preparation:**

- For the processing of NucleoProtect® VET-stabilized swab samples, additional buffer PFN is needed (REF 740121.5, see ordering information).
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature (18 – 25 °C).
- Preheat RNase-free $H_2O$ (for elution) to 70 °C.

| | | |
|---|---|---|
| **1** | **Sample lysis** | |
| | Vortex the NucleoProtect® VET Blood tube or the tube containing a blood sample stabilized in NucleoProtect® VET reagent thoroughly for 30 seconds. Briefly centrifuge the tube for 10 sec. at 200 x *g* to remove drops form inside the lid. | **transfer 200 µL solution into fresh tube** |
| | Transfer **200 µL of the solution** (containing approximately 57 µL blood) and transfer it into a fresh tube (e.g. 1.5 mL). | |
| | Add **100 µL Lysis Buffer SVL, 100 µL water, 2 µL Buffer PFN** and vortex for 10 – 15 s. | **+100 µL SVL +100 µL water +2 µL PFN** |
| | *Note: Buffer SVL, water and PFN can be premixed in a Mastermix. For 10 samples, mix 1000 µL SVL, 1000 µL water and 20 µL PFN.* | |
| | Add **10 µL of Liquid Proteinase K** into the tube and mix moderately. | **+10 µL Liquid Proteinase K** |
| | Mix the tube for **10 min** at **1,400 rpm** (e.g. on an Eppendorf Thermoshaker) at room temperature. | |
| | Add **2.5 µL Carrier-RNA working solution** (100 ng/µL) to the tube. | **opt.: +2.5 µL Carrier RNA** |
| | Mix the tube content by vortexing or pipetting up and down. | **Mix** |
| | Incubate for 3 min at room temperature. | **RT, 3 min** |
| | If necessary, briefly centrifuge the tube (~ 1 s at ~ 2.000 xg) to remove drops from the lid (short spin only). | **~ 1 s, ~ 2,000 x *g*** |
| **2** | After sample pretreatment, continue with the standard protocol procedure in section 5.1 step 2 (page 11): Adjust binding conditions (which is the addition of 200 µL ethanol). | |

# 6 FastTrack protocol for plasma, serum and swab wash solutions

Some sample materials, like plasma, serum, swab wash solutions, and other cell-free biological fluids allow preparation with a high focus on ease of handling and speed of preparation according to the following fast-track protocol. Yield of nucleic acid may be reduced, depending on sample material and analyte (RNA, DNA).

For more complex or inhibitor-rich sample matrices like blood, feces, and tissue, we recommend to follow the standard procedures described in chapter 5.

Before starting the preparation:

- For frozen plasma and serum samples we recommend not to freeze-thaw more than once before processing.
- Check if Wash Buffer SVW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (working solution).
- The complete procedure should be performed at room temperature.
- A heat incubator is not required.

**Set-up proteinase – Carrier RNA premix**

Depending on the number of preparations to be processed, prepare a Liquid Proteinase – Carrier-RNA premix according to the following table. After set-up of the premix, keep it on ice and use it within one hour.

| Number of preps | Volume of Liquid Proteinase K | Volume of Carrier RNA |
|---|---|---|
| 1 | 10 µL | 2.5 µL |
| 2 | 22 µL | 5.5 µL |
| 3 | 33 µL | 8.25 µL |
| 4 | 44 µL | 11 µL |
| 5 | 55 µL | 13.75 µL |
| 6 | 66 µL | 16.5 µL |
| 7 | 77 µL | 19.25 µL |
| 8 | 88 µL | 22 µL |
| 9 | 99 µL | 24.75 |
| 10 | 110 µL | 27.5 µL |
| 11 | 121 µL | 30.25 µL |
| 12 | 132 µL | 33 µL |

**1 Lyse sample**

Pipet **12.5 µL Liquid Proteinase K – Carrier-RNA Mix** in a Collection Tube (1.5 mL, provided).

Add **200 µL sample** to the tube and mix moderately.

Add **200 µL Lysis Buffer SVL** to the tube.

Mix the tube for **10 min at 1,400 rpm** (e.g. on an Eppendorf Thermoshaker) at room temperature.

If neccesary, briefly **centrifuge** the Collection Tube **(~ 1 s** at **~ 2,000 x *g*)** to remove drops from the lid (short spin only).

**2 Adjust binding conditions**

Add **200 µL ethanol** (96 – 100 %) to the tube and mix by vortexing (10 – 15 s).

Incubate for **2 min** at **room temperature**.

Briefly **centrifuge** the Collection Tube (**~ 1 s** at **~ 2,000 x *g***) to remove drops from the lid (short spin only).

**3 Bind viral RNA / DNA**

**Load the lysate (approx 610 µL)** onto a NucleoSpin® VET Column.

Centrifuge **1 min** at **11,000 x *g***.

Place the NucleoSpin® VET Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

**4 Wash and dry silica membrane**

Add **400 µL Wash Buffer SVW1** to the NucleoSpin® VET Column.

**Centrifuge 1 min** at **11,000 x *g***.

Place the NucleoSpin® VET Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

Add **200 µL Wash Buffer SVW2** to the NucleoSpin® VET Column.

Centrifuge **1 min 11,000 x *g***.

Place the NucleoSpin® VET Column in a clean Elution Tube (1.5 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

**5 Elute RNA / DNA**

Add **100 µL RNase-free $H_2O$** onto the column.

Incubate **1 min at RT**

Centrifuge **1 min 11,000 x *g***

Keep eluted RNA / DNA on ice or freeze for storage.

# 7 Appendix

## 7.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Small amounts or no viral nucleic acids in the eluate | *Problems with Carrier RNA*<br>• Carrier RNA was not added.<br>*Viral nucleic acids degraded*<br>• Samples should be processed immediately. Ensure appropriate storage conditions up to the processing.<br>• Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Buffer SVL, Carrier RNA, and RNase-free water. |
| Problems with subsequent detection | *Reduced sensitivity*<br>• Change the volume of eluate added to the PCR / RT-PCR.<br>*Ethanol carry-over*<br>• Prolong centrifugation steps in order to remove Buffer SVW2 completely.<br>*Carrier RNA interference with detection method*<br>• Check if Carrier RNA interferes the detection method. Some detection methods tolerate only limited amounts of carrier RNA. |
| General problems | *Clogged membrane*<br>• Centrifuge plasma lysate before the addition of ethanol and subsequent loading onto the corresponding NucleoSpin® VET Columns. |

## 7.2 Ordering information

| Product | REF | Pack of |
|---|---|---|
| NucleoSpin® VET | 740842.10 / .50 / .250 | 10 / 50 / 250 |
| Buffer PFN | 740121.5 | 5 mL |
| NucleoMag® VET | 744200.1 / .4 /.100 | 1 × 96 / 4 × 96 / 100 x 96 |
| NucleoProtect® VET stabilization and inactivation reagent | 740750.50<br>740750.500 | 50 mL<br>500 mL |
| NucleoProtect® VET Blood Tubes | 740755 | 50 |
| Secondary sealing caps for NucleoProtect® VET Blood Tubes | 740756 | 100 |
| NucleoProtect® VET Swab Tubes | 740760 | 50 |
| Secondary sealing caps for NucleoProtectR VET Swab Tubes | 740761 | 100 |
| Collection Tubes (2 mL) | 740600 | 1000 |
| MN Bead Tubes Type D | 740814.50 | pack of 50 |

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

## 7.3 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 customer and are not a part of a contract of sale or of this warranty.

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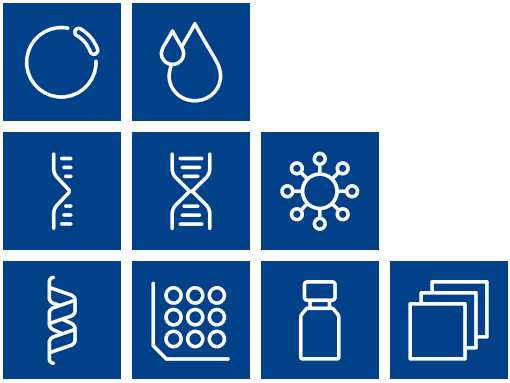

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MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11
52355 Düren · Germany

DE Tel.: +49 24 21 969-0 info@mn-net.com
CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com
FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com
US Tel.: +1 888 321 62 24 sales-us@mn-net.com


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