

# User manual – NucleoProtect® VET stabilization and inactivation reagent

## 1 Components

### 1.1 Product content

REF	NucleoProtect® VET stabilization and inactivation reagent
740750.50 / .500	NucleoProtect® VET stabilization and inactivation reagent for filling into own transport or storage vessels, bottle of 50 mL / 500 mL, User manual

It is strongly recommended to read the protocol details in this user manual when using NucleoProtect® VET stabilization and inactivation reagent for the first time.

All technical literature is available online at [www.mn-net.com](http://www.mn-net.com).

### 1.2 Consumables and Equipment to be supplied by the user

- Depending on the type of specimen to be collected, appropriate leak-proof screw cap tubes, swabs, blood collection tubes or cannulas.
- Personal protection equipment (e.g., lab coat, gloves, goggles).

## 2 Product description

### 2.1 The basic principle

The NucleoProtect® VET stabilization and inactivation reagent is intended for the collection of blood or swab specimens from animals, inactivate viral pathogens and preserve viral nucleic acids (DNA and RNA) by inactivating nucleases. The NucleoProtect® VET stabilization and inactivation reagent allows the preservation and safe transport of veterinary whole blood or swab samples from the site of sample collection site to the analyzing laboratory.

The NucleoProtect® VET stabilization and inactivation reagent allows the safe collection and transport of whole blood or swab specimens from animals and subsequent viral nucleic acid (RNA/DNA) extraction for use in downstream analyses such as (RT)-PCR or q(RT)-PCR.

### 2.2 Product specification

The NucleoProtect® VET reagent was tested with animal blood collected with NucleoProtect® VET Blood Tubes (REF 740755) and EDTA-anticoagulated animal blood. The user is responsible to validate other anticoagulants used in conjunction with the NucleoProtect® VET reagent. The reagent was also tested with different swab materials including foam tip swabs, cotton fibers, synthetic fibers and flocked swab. The user is responsible to validate the individual swab used in conjunction with the NucleoProtect® VET reagent. The product is not intended for human use. The product is not intended for human use.

### Inactivation of viruses

The inactivating of high risk viruses was tested in different background matrices (see table below) using the Tissue Culture Infectious Dose (TCID) assay. Viruses were spiked into the different matrices with titers between  $10^6$  and  $10^7$  virus particles per mL and incubated for 30 minutes in the NucleoProtect® VET reagent. Samples were diluted to prevent toxic effects of the NucleoProtect® VET reagent on cell culture cells and virus specific cell culture cells were infected with the treated viruses. Culture cells were subsequently incubated several days and analyzed for cytopathic effects (CPE). An aliquot of each cell culture supernatant was then passaged to a fresh cell culture. In case of a lack of viral growth in both cell culture infection assays the viruses were considered as completely inactivated. The titer reduction was calculated from the starting titer and the dilution factor.

The level of virus titer reduction is shown in the table below (data kindly provided by the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health of Germany).

Virus	Background Matrix	Reduction of virus titer
Foot-and-mouth disease virus (FMDV)	Bovine tongue epithelium Whole blood Raw milk Saliva	> $10^4$ (corresponds to an inactivation capacity of > 99,99 %)
Bluetongue virus (BTV-5)		
Peste des petits ruminants virus (PPRV)		
Lumpy skin disease virus (LSDV)		
Bovine Coronavirus (BCoV)	Saliva	

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.

### Sample stability

The stabilization capability of the NucleoProtect® VET reagent was tested with all naturally occurring viral genome structures: ss (+/-)RNA, dsRNA and dsDNA. In particular, the following viruses were tested:

Virus	Genome structure
Foot-and-mouth disease virus (FMDV) Porcine enterovirus (PEV) Bovine viral diarrhea virus (BVDV)	ss(+)/RNA
Peste des petits ruminants virus (PPRV) Canine distemper virus (CDV)	ss(-)/RNA
Bluetongue virus (BTV serotypes 5 and 8)	dsRNA
Lumpy skin disease virus (LSDV) Vaccinia virus (VV) Bovine herpes Virus (BoHV)	dsDNA

Stabilization of viral RNA and DNA was tested with spiked blood, or swab samples using virus specific q(RT)-PCR assays. NucleoProtect® VET-stabilized samples (swabs/blood) can be stored and shipped at the following conditions without sensitivity loss\*:

Storage condition	Storage time*
15 – 25 °C	7 days
4 °C	7 days
37 °C	2 days
-20 °C	30 days

\* the specified storage time indicates how long the samples can be stored at the specified condition with a change in C<sub>q</sub>-value of < 1 on average, as demonstrated in q(RT)-PCR analyses comparing stored samples to the time point of collection.

The NucleoProtect® VET reagent is not intended for preserving morphology or viability of microorganisms. Due to its lytic and denaturing activity, the NucleoProtect® VET reagent is not suited for serological testing.

### 2.3 Handling of sample material

The NucleoProtect® VET reagent is intended for safe collection and transport of animal whole blood or swab samples only.

The NucleoProtect® VET stabilization reagent appears colorless and clear. Do not use NucleoProtect® VET reagent if the solution appears discolored or contains precipitates.

Do not use if the product or packaging is damaged or if there is evidence of leakage.

Recommended NucleoProtect® VET reagent volume for different sample types.

Sample	Recommended Volume
Animal whole blood	Add 2.5 volumes of NucleoProtect® VET reagent per volume of animal whole blood. Calculate the required NucleoProtect® VET reagent volume accordingly for individual blood volume used, e.g.: For 100 µL blood add 250 µL NucleoProtect® VET reagent For 500 µL blood add 1250 µL NucleoProtect® VET reagent For 1.0 mL blood add 2,5 mL NucleoProtect® VET reagent For 1.5 mL blood add 3.75 mL NucleoProtect® VET reagent
Swabs	The amount of NucleoProtect® VET reagent needed for effective sample preservation and inactivation depends on the size and material of the swab (swab head material, such as cotton, or synthetic / inert fibres). We recommend adding enough NucleoProtect® VET reagent to submerge the swab material. Ensure that the swab material is compatible with the NucleoProtect® VET reagent.

The user is responsible to use leak proof container in conjunction with the NucleoProtect® VET reagent.

The NucleoProtect® VET Blood Tube is intended for professional use only. Follow veterinarian and laboratory's standard procedures for collecting blood or swab samples from animals and apply appropriate safety procedures.

### Collecting Swab samples

Insert Swab into the NucleoProtect® VET reagent after collecting the veterinary sample. Do not insert the swab into the NucleoProtect® VET reagent before collecting the sample!

### Collecting Blood samples

Follow veterinary and laboratory standard procedures for collecting blood from animals and apply appropriate safety practices when collecting blood. If other primary blood collection tubes than EDTA-tubes are used to collect blood from animals, the user is responsible to validate the use in conjunction with the NucleoProtect® VET reagent.

## 3 Product storage conditions

Store NucleoProtect® VET reagent at ambient temperatures (15 – 25 °C).

The product can be used until the expiry date printed on the label.

## 4 Safety instructions

Always wear suitable protective clothing (e.g., protective clothes, gloves, and goggles) when working with the NucleoProtect® VET reagent or when working with potentially infectious biological material.

For more information consult the appropriate Material Safety Data Sheets (MSDS available online at [www.mn-net.com/msds](http://www.mn-net.com/msds)).



Avoid mixing the NucleoProtect® VET reagent with sodium hypochlorite (bleach) or other strong acids and bases. The mixtures could form highly reactive compound when combined with bleach!

The NucleoProtect® VET reagent contains chemical additives. Prevent contact with the animal.

Remove spilled NucleoProtect® VET reagent with an excess of water.

## 5 Nucleic acid extraction

The NucleoProtect® VET reagent is compatible with the MACHEREY-NAGEL NucleoSpin® VET kit (silica membrane single spin kit; REF 740842.10 / .50 / .250) and the NucleoMag® VET kit (magnetic bead based kit; REF 744200.1 / .4) nucleic acid extraction systems.

The NucleoProtect® VET reagent is also compatible with other common commercial nucleic acid extraction solutions. The user is responsible for validating additional nucleic acid extraction and purification methodologies.

### Nucleic acid extraction using the NucleoMag® VET\* kit (see ordering information below):

For detailed description of the nucleic acid extraction procedure from NucleoProtect® VET-stabilized samples using the NucleoMag® VET kit, please refer to the user manual.

Download user manual NucleoMag® VET\*:



[www.mn-net.com/NucleoMagVETmanual](http://www.mn-net.com/NucleoMagVETmanual)

Carefully read the user manual of the NucleoMag® VET kit before starting the nucleic acid extraction procedure.

For the processing of blood collected with the NucleoProtect® VET reagent using the NucleoMag® VET kit, additional buffer VIA is needed (REF 744206, see ordering information).

Non-stabilized and NucleoProtect® VET-stabilized samples can be processed in parallel. For detailed description, please refer to the user manual.

**\*The NucleoMag® VET extraction kit has been automated on a wide variety of open liquid-handling or magnetic-rod platforms. Please contact MACHEREY-NAGEL for technical automation support and verified methodologies: tech-bio@mn-net.com | +49 24 21 969-270**

### Nucleic acid extraction using the NucleoSpin® VET kit (see ordering information below):

For detailed description of the nucleic acid extraction procedure from NucleoProtect® VET-stabilized samples using the NucleoSpin® VET kit, please refer to the user manual.

Download user manual NucleoSpin® VET:



[www.mn-net.com/NucleoSpinVETmanual](http://www.mn-net.com/NucleoSpinVETmanual)

Carefully read the user manual of the NucleoSpin® VET kit before starting the nucleic acid extraction procedure.

For the processing of NucleoProtect® VET-stabilized samples using the NucleoSpin® VET kit, additional buffer PFN is needed (REF 740121.5, see ordering information).

Non-stabilized and NucleoProtect® VET-stabilized samples can be processed in parallel. For detailed description, please refer to the user manual.

## 6 Disposal

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

Discard blood collection sharps like cannulas in biohazard containers approved for their disposal. Please follow local waste regulations.

## 7 Contact information

For more detailed product use restriction / warranty please visit: [www.mn-net.com](http://www.mn-net.com)

Please contact our technical support for any product related inquiries:

MACHEREY-NAGEL Germany Tel.:

+49 (0) 24 21 969-270

e-mail: tech-bio@mn-net.com

## 8 Ordering information

Product	Description	Pack of	REF
NucleoProtect® VET stabilization and inactivation reagent	NucleoProtect® VET stabilization and inactivation reagent for filling into own transport or storage vessels	50 / 500 mL	740750.50 / 500
<b>Accessory / related products</b>			
NucleoProtect® VET Blood Collection Tubes	Blood collection tube prefilled with 4.0 mL NucleoProtect® VET stabilization and inactivation reagent, compatible with standard Luer cannulas, box of 50 tubes	50	740755
NucleoProtect® VET Swab Tube	Screw cap swab collection tube prefilled with 1.5 mL NucleoProtect® VET stabilization and inactivation reagent including swabs	50	740760
Secondary caps for NucleoProtect® VET Blood Tubes Tubes	Sealing caps for NucleoProtect® VET Blood Tubes	100	740756
Secondary caps for NucleoProtect® VET Swab Tubes	Sealing caps for NucleoProtect® VET Swab Tubes	100	740761
NucleoMag® VET	Magnetic bead based kit for extraction of RNA and DNA from veterinary samples, containing NucleoMag® B-Beads, buffers, Carrier RNA, Proteinase K	1 × 96 / 4 × 96	744200.1 / .4
Buffer VIA	Needed for the nucleic acid extraction from NucleoProtect® VET collected blood samples using the NucleoMag® VET kit	150 mL	744206
NucleoSpin® VET	Single spin kit for extraction of RNA and DNA from veterinary samples, containing binding columns, collection Tubes (2 mL), collection tubes for lysis and elution (1.5 mL), buffers, liquid Proteinase K, Carrier RNA (lyophilized)	10 / 50 / 250	740842.10 / .50 / .250
Buffer PFN	Needed for the nucleic acid extraction from NucleoProtect® VET collected samples using the NucleoSpin® VET kit	5 mL	740121.5

Visit [www.mn-net.com](http://www.mn-net.com) for more detailed product information or contact our technical support (tech-bio@mn-net.com)

## 9 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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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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