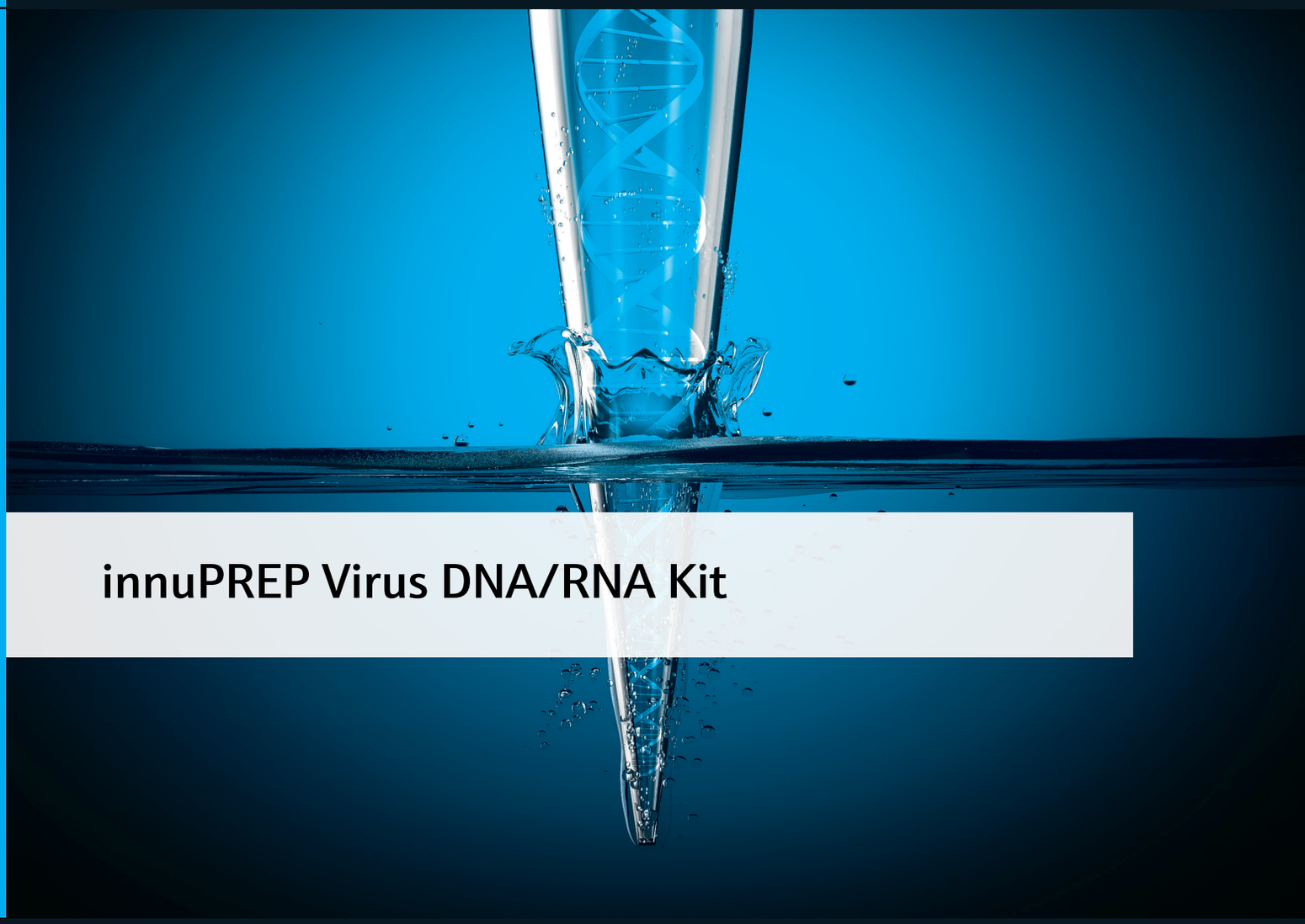
Instructions for Use
Life Science Kits & Assays



innuPREP Virus DNA/RNA Kit

Innuscreen GmbH

Order No.:

845-KS-4800010 10 reactions
845-KS-4800050 50 reactions
845-KS-4800250 250 reactions

Publication No.: HB_KS-4800_e_220201

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Contents

1 Introduction

1.1 Intended use

The innuPREP Virus DNA/RNA Kit has been designed for isolation of viral DNA and RNA from different kinds of starting material. The extraction procedure is based on a new kind of chemistry (patent pending). The innuPREP Virus DNA/RNA Kit is optimized for the rapid preparation of highly pure viral nucleic acids from cell free fluid biological samples, for example: plasma, serum, urine, liquor as well as cell culture supernatant, solid materials and swabs.

The procedure combines lysis of starting material with subsequent binding of viral nucleic acids onto the surface of a Spin Filter membrane. After several washing steps the viral nucleic acids are eluted from the membrane by using RNase-free water. Extraction chemistry and extraction protocol are optimized to get maximum yield. Further, the kit contains a Carrier Mix with Carrier RNA as well as an internal control DNA and RNA. The internal control DNA and RNA can be used in combination with a corresponding real-time PCR detection kit (innuDETECT Internal Control DNA/RNA Assay).

The kit works with 200 µl and 400 µl liquid, tissue and swab samples. The extracted viral nucleic acids are suitable for downstream applications like PCR, real-time PCR or any kind of enzymatic reaction.

The detection limit for certain viruses depends on the individual procedures, for example in-house PCR or commercial used detection assays.

We highly recommend the usage of the internal control DNA or RNA (IC DNA/RNA) or own internal standards (low copy) respectively, as well as positive and negative controls to monitor the purification, amplification, and detection processes.

Please note that the eluates contain both viral nucleic acids and Carrier RNA. In case of using Carrier RNA the quantification of nucleic acids (isolated with this kit) by photometric or fluorometric methods is not possible. It is recommended to quantify extracted DNA and RNA with other methods like specific quantitative PCR or real-time PCR. Furthermore, Carrier RNA may inhibit PCR reactions. Thus, the amount of added Carrier

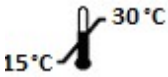
RNA has to be carefully optimized depending on the individual PCR system used.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains sufficient reagents for <N> reactions.
	**Storage conditions** Store at room temperature or shown conditions respectively.
	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	**Expiry date**
	**Lot number** The number of the kit charge.
	**Manufactured by** Contact information of manufacturer.
	**For single use only** Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → “Notes on the use of this manual" p.4).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before use to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles to avoid any injuries. IST Innuscreen GmbH has not tested the liquid waste generated during use of the kit for potential residual infectious components. This case is highly unlikely but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample prepa-

ration!

NOTE
Emergency medical information in English and German can be obtained 24 hours a day from:
Poison Information Center, Freiburg / Germany
Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

All components of the kit are shipped at ambient temperature.

Store lyophilized and dissolved Proteinase K at 4 °C to 8 °C.

All other components of the innuPREP Virus DNA/RNA Kit should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

Before every use make sure that all components are at room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This kit was produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP Virus DNA/RNA Kit or other products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 30 9489 3380. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those referred to in the manual (→ “Intended use”, p.2) (→ “Product specifications”, p.13). Since the performance characteristics of IST Innuscreen GmbH kits have only been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA ´88 regulations in the U.S. or equivalent regulations required in other countries.

All products sold by IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50	Σ 250
REF	845-KS-4800010	845-KS-4800050	845-KS-4800250
Lysis Solution CBV	5 ml	25 ml	120 ml
Binding Solution SBS	15 ml	60 ml	250 ml
Carrier Mix	1 x lyophilized powder	1 x lyophilized powder	3 x lyophilized powder
RNase-free water	2.0 ml	2.0 ml	3 x 2.0 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution	for 4 x 1.5 ml working solution
Washing Solution HS (conc.)	5 ml	15 ml	70 ml
Washing Solution LS (conc.)	6 ml	16 ml	2 x 36 ml
RNase-free water	2.0 ml	2 x 2.0 ml	25 ml
Spin Filter (blue)	10	50	5 x 50
Receiver Tubes (2.0 ml)	50	5 x 50	25 x 50
Elution Tubes (1.5 ml)	10	50	5 x 50
Manual	1	1	1

6.2 Components not included in the kit

- 1.5 ml and 2.0 ml reaction tubes
- 96-99.8% ethanol (molecular biology grade, undenatured)
- ddH_2O for dissolving Proteinase K

7 General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases must be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free).
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven-baked at 240 °C for four or more hours before use. Autoclaving alone will not inactivate many RNases completely. Oven-baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C and then it has to be autoclaved or heated to 100 °C for 15 min to remove residual DEPC.

- Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free ddH2O.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

8 Carrier Mix

8.1 Storage conditions and handling

The Carrier Mix contains a carrier RNA and an internal control DNA and RNA (IC DNA/RNA).

- Add dissolved Carrier Mix to Lysis Solution CBV immediately.
- Unused Carrier Mix should be kept frozen at −20 °C.
- Do not freeze and thaw the Carrier Mix more than 3 times.
- Mixture of Lysis Solution CBV/Carrier Mix is stable for 1 day at 4 °C.

Internal control DNA and RNA can be detected by real-time PCR using the corresponding assay:

Name	Amount	Order No.
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

8.2 Preparation of Lysis Solution CBV / Carrier Mix

Add 1.25 ml RNase-free water to each tube of Carrier Mix. Mix thoroughly by pipetting up and down.
Prepare mixture of **Lysis Solution CBV** and **Carrier Mix** according to the tables below.

For extraction from **200 µl** of serum/plasma or other cell-free body fluids:

Component	5 samples	10 samples	n samples
Lysis Solution CBV	1.2 ml	2.4 ml	240 µl x sample
Carrier Mix	60 µl	120 µl	12 µl x sample
Final volume	1.26 ml	2.52 ml	252 µl x sample

For extraction from **400 µl** of serum/plasma or other cell-free body fluids:

Component	5 samples	10 samples	n samples
Lysis Solution CBV	2.4 ml	4.8 ml	480 µl x sample
Carrier Mix	60 µl	120 µl	12 µl x sample
Final volume	2.46 ml	4.92 ml	492 µl x sample

If customized extraction controls are used, please add these components to the mixture of **Lysis Solution CBV / Carrier Mix**.

9 Product Specifications

1. Starting material
   - Serum, plasma, cell culture supernatants and other cell-free body fluids (200 µl and 400 µl)
   - Tissue samples and biopsies (max. 20 mg)
   - Swab samples

NOTE
Avoid freezing and thawing of starting material!

2. Time for isolation

   Approximately 25 minutes

3. Positive tested for

   E.g., HBV, CMV, EBV, HSV, FMDV

10 Initial steps before starting

- Add the indicated amount of absolute ethanol to each bottle **Washing Solution HS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-4800010	Add 5 ml ethanol to 5 ml Washing Solution HS (conc.).
845-KS-4800050	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
845-KS-4800250	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

- Add the indicated amount of absolute ethanol to each bottle **Washing Solution LS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-4800010	Add 24 ml ethanol to 6 ml Washing Solution LS (conc.).
845-KS-4800050	Add 64 ml ethanol to 16 ml Washing Solution LS (conc.).
845-KS-4800250	Add 144 ml ethanol to 36 ml Washing Solution LS (conc.).

- Add the indicated amount of ddH2O to the **Proteinase K**, mix thoroughly and store as described above.

845-KS-4800010	Add 0.3 ml ddH2O to lyophilized Proteinase K.
845-KS-4800050	Add 1.5 ml ddH2O to lyophilized Proteinase K.
845-KS-4800250	Add 1.5 ml ddH2O to lyophilized Proteinase K.

- Prepare mixture of **Lysis Solution CBV** and **Carrier Mix** as described (→ see chapter 8.2)
- Centrifugation steps should be carried out at room temperature.
- Heat thermal mixer or water bath to 70°C
- Pre-heat RNase-free water to 70°C

NOTE
Do not use pre-heated RNase-free water for Carrier Mix.

11 Protocol 1: Isolation of viral DNA/RNA from serum, plasma, cell culture supernatants/mediums or cell-free body fluids up to 200 µl

IMPORTANT

Pre-fill the needed amount of RNase-free water into a 1.5 ml reaction tube and incubate the RNase-free water at 70 °C until the elution step.

Prepare Lysis Solution CBV / Carrier Mix as described in chapter 8.2 before starting.

1. Pipette **200 µl Lysis Solution CBV / Carrier Mix** into a 2.0 ml reaction tube. Add **200 µl of the sample and 20 µl of Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3 – 4 times during incubation. No shaking will reduce the lysis efficiency.

After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.

2. Add **400 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

3. Apply the sample to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver tube with the filtrate. Place the Spin Filter into a a new 2.0 ml Receiver Tube.

4. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

5. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

6. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

7. Centrifuge at 10.000 x g (~12.000 rpm) for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heated RNase-free water** (70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free water (e.g. 30 µl + 30 µl) might increase the yield of extracted viral DNA/RNA.

NOTE
The viral DNA/RNA can be eluted with a lower or a higher volume of RNase-free water (depends on the expected yield of viral DNA/RNA). Elution with lower volumes of RNase-free water increases the final concentration of viral DNA/RNA. Store the extracted viral DNA/RNA at +4 °C. For long time storage placing at −22 °C to −18 °C is recommended.

12 Protocol 2: Isolation of viral DNA/RNA from serum, plasma, cell culture supernatants/mediums or cell-free body fluids up to 400 µl

IMPORTANT

Pre-fill the needed amount of RNase-free water into a 1.5 ml reaction tube and incubate the RNase-free water at 70 °C until the elution step.

Prepare Lysis Solution CBV / Carrier Mix as described in chapter 8.2 before starting.

1. Pipette **400 µl Lysis Solution CBV / Carrier Mix** into a 2.0 ml reaction tube. Add **400 µl of the sample and 20 µl of Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3 – 4 times during incubation. No shaking will reduce the lysis efficiency.

After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.

2. Add **800 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

3. Apply 650 µl of the sample to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver tube with the filtrate. Place the Spin Filter into a a new 2.0 ml Receiver Tube.

4. Place the Spin Filter into a new 2.0 ml Receiver Tube and load the residual sample onto the Spin Filter. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 min.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver tube with the filtrate. Place the Spin Filter into a a new 2.0 ml Receiver Tube.

5. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

6. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

7. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

8. Centrifuge at 10.000 x g (~12.000 rpm) for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

9. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heated RNase-free water** (70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free water (e.g. 30 µl + 30 µl) might increase the yield of extracted viral DNA/RNA.

NOTE

The viral DNA/RNA can be eluted with a lower or a higher volume of RNase-free water (depends on the expected yield of viral DNA/RNA). Elution with lower volumes of RNase-free water increases the final concentration of viral DNA/RNA. Store the extracted viral DNA/RNA at +4 °C. For long time storage placing at −22 °C to −18 °C is recommended.

13 Protocol 3: Isolation of viral DNA/RNA from tissue samples or biopsies (max. 20 mg)

IMPORTANT

Pre-fill the needed amount of RNase-free water into a 1.5 ml reaction tube and incubate the RNase-free water at 70 °C until the elution step.

Prepare Lysis Solution CBV / Carrier Mix as described in chapter 8.2 before starting.

1. Cut max. **20 mg of the sample** into small pieces and prepare a 10 % (w / v) suspension of tissue in buffer (e.g. RNase-free water or PBS) using commercial homogenization tools (bead-based homogenization or other homogenization tools).

2. Centrifuge the suspension at max. speed for 2 minutes to remove particles. Use the clear particle-free supernatant for further processing.

3. Add **200 µl Lysis Solution CBV / Carrier Mix** into a 1.5 ml reaction tube, add **200 µl of the sample and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3 – 4 times during the incubation. No shaking will reduce the lysis efficiency.

After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.

4. Apply **400 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

5. Apply the sample to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver tube with the filtrate. Place the Spin Filter into a a new 2.0 ml Receiver Tube.

6. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

7. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

8. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

9. Centrifuge at 10.000 x g (~12.000 rpm) for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

10. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heated RNase-free water** (70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free water (e.g. 30 µl + 30 µl) might increase the yield of extracted viral DNA/RNA.

Protocol 3: Isolation of viral DNA/RNA from tissue samples or biopsies (max. 20 mg)

NOTE

The viral DNA/RNA can be eluted with a lower or a higher volume of RNase-free water (depends on the expected yield of viral DNA/RNA). Elution with lower volumes of RNase-free water increases the final concentration of viral DNA/RNA. Store the extracted viral DNA/RNA at +4 °C. For long time storage placing at −22 °C to −18 °C is recommended.

14 Protocol 4: Isolation of viral DNA/RNA from swabs

IMPORTANT

Pre-fill the needed amount of RNase-free water into a 1.5 ml reaction tube and incubate the RNase-free water at 70 ℃ until the elution step.

Prepare Lysis Solution CBV / Carrier Mix as described in chapter 8.2 before starting.

1. Place the swab into a 1.5 ml reaction tube containing physiological saline (0,9 % NaCl) and incubate for 15 minutes at room temperature. Afterwards shake the swab vigorously, squeeze it and remove the swab. Proceed with **200 µl of the particle-free sample** for further steps

2. Add **200 µl Lysis Solution CBV / Carrier Mix** into a 1.5 ml reaction tube, add **200 µl of the sample and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 ℃ for 10 minutes.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3 – 4 times during the incubation. No shaking will reduce the lysis efficiency.

After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.

3. Apply **400 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

4. Apply the sample to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver tube with the filtrate. Place the Spin Filter into a a new 2.0 ml Receiver Tube.

5. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

6. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

7. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

8. Centrifuge at 10.000 x g (~12.000 rpm) for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

9. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heated RNase-free water** (70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free water (e.g. 30 µl + 30 µl) might increase the yield of extracted viral DNA/RNA.

NOTE

The viral DNA/RNA can be eluted with a lower or a higher volume of RNase-free water (depends on the expected yield of viral DNA/RNA). Elution with lower volumes of RNase-free water increases the final concentration of viral DNA/RNA. Store the extracted viral DNA/RNA at +4 °C. For long time storage placing at −20 °C is recommended.

15 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
Low amount of extracted viral DNA/RNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer to 5 minutes or repeat elution step once again. Take a higher volume of RNase-free water.
Insufficient mixing with Binding Solution SBS	Mix sample with Binding Solution SBS by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted viral DNA/RNA	
Too much RNase-free water	Elute the viral DNA/RNA with lower volume of RNase-free water.
No Carrier RNA added	Add Carrier RNA (Carrier Mix) to the sample, as described in the manual above.

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