Instructions for Use
Life Science Kits & Assays

innuPREP Virus TS RNA Kit

For research use only !

analytikjena
An Endress+Hauser Company

1 Introduction

1.1 Intended use

The **innuPREP Virus TS RNA Kit** has been designed for fast isolation of viral RNA from tracheal swabs. The extraction procedure is based on a new kind and patented chemistry.

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 of yield. Further, the kit contains a Carrier RNA.

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 own 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 (see related products).

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 RNA with other methods like specific quantitative PCR or real-time PCR. Furthermore, Carrier RNA may inhibit PCR reactions. Thus the amount of add 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 prior to 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.

2 Safety precautions

NOTE
Read through this chapter carefully prior 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 personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulations.

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 preparation!

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 please download the Safety Data Sheet (SDS) from our website (www.analytik-jena.com). Find the SDS of each product and its components in the “Downloads”-folder.

3 Storage conditions

All kit components are shipped at ambient temperature. Upon arrival, store lyophilized **Carrier RNA** at -22 °C to -18 °C. Divide dissolved **Carrier RNA** into aliquots and store at -22 °C to -18 °C. Do not freeze and thaw more than 3 times.

Store lyophilized **Proteinase K** at 4 °C to 8 °C. Aliquot dissolved **Proteinase K** and store at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!

All other components of the innuPREP Virus TS 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.

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

4 Functional testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. This product has been 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 TS RNA Kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. 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 (→ "Product specifications" p. 5). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols than those described below. Analytik Jena AG 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 equivalents in other countries.

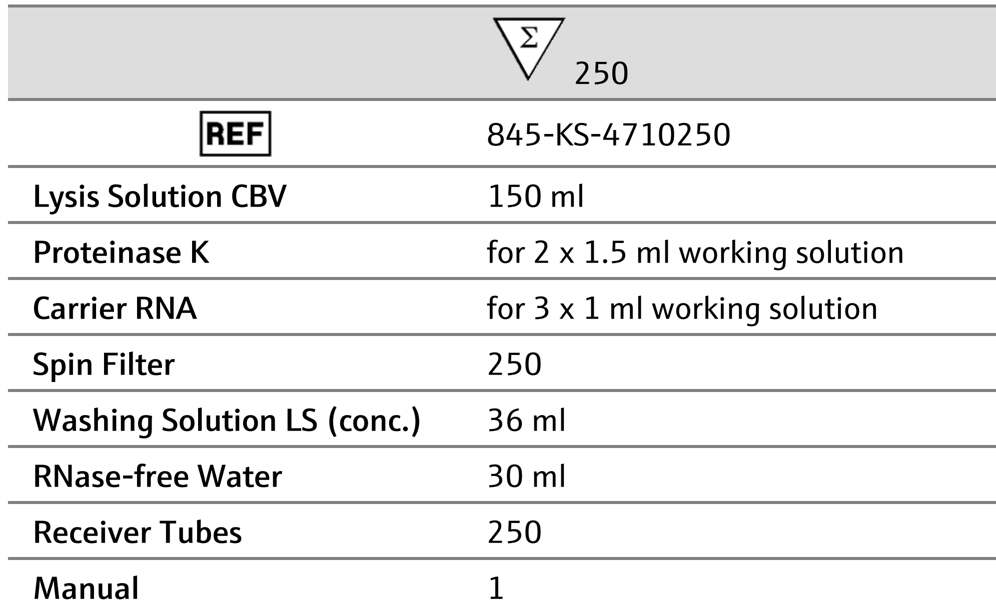
All products sold by Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE

For research use only!

6 Kit components

6.1 Components included in the kit



	Σ 250
REF	845-KS-4710250
Lysis Solution CBV	150 ml
Proteinase K	for 2 x 1.5 ml working solution
Carrier RNA	for 3 x 1 ml working solution
Spin Filter	250
Washing Solution LS (conc.)	36 ml
RNase-free Water	30 ml
Receiver Tubes	250
Manual	1

6.2 Components not included in the kit

- 1.5 ml and 2.0 ml tubes
- 80 % ethanol (molecular biology grade, undenaturated)
- 2-Propanol (molecular biology grade)
- ddH_2O for dissolving **Proteinase K**

7 Product specifications

1. Starting material:

- Swab samples

2. Time for isolation:

- Approximately 25 minutes

8 Initial steps before starting

- Add to each vial of lyophilized **Proteinase K** 1.5 ml ddH_2O, mix thoroughly and store as described above.
- Add to each vial of **Carrier RNA** 1 ml RNase-free Water, mix thoroughly and store as described above.
- Add to **Washing Solution LS (conc.)** 144 ml absolute ethanol and mix thoroughly. Keep the bottle always firmly closed!
- Avoid freezing and thawing of starting material.
- Pre-heat thermal mixer or water bath to 70 °C
- Pre-heat RNase-free Water to 70 °C
- centrifugation steps should be carried out at room temperature

9 Protocols for isolation of viral RNA

NOTE
Pre-fill needed amount of RNase-free Water in a 1.5 ml reaction tube and pre-heat to 70 °C until the elution step.

9.1 Protocol 1: Isolation from swabs stored under physiological saline in a storage tube

1. Open the storage tube with the physiological saline. Shake the swab vigorously, squeeze it as complete as possible and remove the swab. Proceed with **200 µl of the particle-free sample** for further steps.
2. Add **200 µl Lysis Solution CBV** and **10 µl Carrier RNA** into a 1.5 ml reaction tube.
3. Add **200 µl of the sample** and **10 µl Proteinase K** to each well used. Mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

NOTE
We recommend to use 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!

4. After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.
5. Add **400 µl isopropanol** to the lysed sample, mix by vortexing or by pipetting up and down several times.

NOTE
It is important that the sample and isopropanol are mixed vigorously to get a homogeneous solution.

6. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 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.

7. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

8. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.

9. Open the Spin Filter and add **650 µl 80 % Ethanol**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.

10. Open the Spin Filter and add **650 µl 80 % Ethanol**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.

11. Centrifuge at 11,000 x g (~11,000 rpm) for 4 min to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

12. Place the Spin Filter into a 1.5 ml Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heat RNase-free Water** (70°C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~11,000 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 RNA.

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

9.2 Protocol 2: Isolation directly from swabs

1. Transfer 6**00 µl Lysis Solution CBV** into a 2.0 ml reaction tube.
2. Incubate the swab 10 minutes insight the tube. Shake the swab vigorously, squeeze it as complete as possible and remove the swab. Proceed with **400 µl of the particle-free sample** for further steps
3. Transfer **400 µl of the sample** into a 1.5 ml tube and add **10 µl Carrier RNA** and **10 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

NOTE
We recommend to use 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!

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4. After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.
5. Add **400 µl isopropanol** to the lysed sample, mix by vortexing or by pipetting up and down several times.

NOTE
It is important that the sample and isopropanol are mixed vigorously to get a homogeneous solution.

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6. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 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.

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7. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
8. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.

9. Open the Spin Filter and add **650 µl 80 % Ethanol**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.

10. Open the Spin Filter and add **650 µl 80 % Ethanol**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.

11. Centrifuge at 11,000 x g (~11,000 rpm) for 4 min to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

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

Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homo-genization	Increase lysis time. Increase centrifugation speed. After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no viral RNA eluted	
Insufficient disruption or homo-genization	Increase lysis time. Reduce amount of starting material. Over-loading reduces yield!
Incomplete elution	Prolong the incubation time with RNase-free Water to 5 minutes or repeat elution step once again. Take a higher volume of RNase-free Water.
Insufficient mixing with isoropanol	Mix sample with isopropanol by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted viral DNA	
Too much RNase-free Water	Elute the viral RNA with lower volume of RNase-free Water.
No Carrier RNA added	Add Carrier RNA to the sample, as de-scribed in the manual above.
Viral RNA does not perform well in downstream applications (e.g. RT-PCR)	
Ethanol carryover during elution	Increase time for removing of ethanol.
Salt carryover during elution	Ensure that **Washing Solution LS** is at room temperature. Checkup Washing Solution for salt precipi-tates. If there are any precipitate dissolves these precipitate by carefully warming.