analytikjena

Manual

innuPREP Virus RNA Kit - KFml

Life Science unlimited

Order No.:

845-KF-4515015	15 reactions
845-KF-4515250	250 reactions
845-KF-4515750	750 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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Contents

1 Introduction

1.1 Intended use

The innuPREP Virus RNA Kit - KFml has been designed for isolation of viral RNA from different kinds of starting material. The kit contains a Carrier Mix with Carrier RNA as well as an internal extraction control for DNA and RNA. The Internal Control DNA or RNA can be detected by real-time PCR using the corresponding assays.

The extraction procedure is based on a new kind of chemistry (patent pending). The procedure combines lysis of starting material with subsequent binding of viral RNA on surface modified magnetic particles. After washing steps the viral RNA is eluted from the magnetic particles by using water.

The extraction procedure takes place on the magnetic particle processor KingFisher ml. Extraction chemistry and extraction protocol are optimized to get maximum yield of RNA.

Consult instruction for use

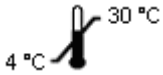
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual

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

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.

Used by
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 device 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 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 personal 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. Analytik Jena AG has not tested the liquid waste generated during using 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. Observe 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, please ask for the material safety data sheet (MSDS):

3 Storage conditions

The innuPREP Virus RNA Kit - KFml should be stored dry, at room temperature (14 °C – 25 °C) and is stable for at least 6 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

4 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. The components of each innuPREP Virus RNA Kit - KFml were tested by recovery of IC RNA spiked in serum in serial dilutions and subsequent real-time PCR.

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 RNA Kit - KFml 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 user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. 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

Important
Store the MAG Suspension at 4 °C – 8 °C.
Store lyophilized Carrier Mix at – 20 °C.

Storage conditions
All other components are stored at room temperature.

	Σ 15	Σ 250	Σ 750
REF	845-KF-4515015	845-KF-4515250	845-KF-4515750
MAG Suspension	1 ml	3x 5,5 ml	7x 5,5 ml
Carrier Mix	1x lyophilized powder	3x lyophilized powder	7x lyophilized powder
RNase-free Water	1x 2 ml	3x 2 ml	7x 2 ml
Lysis Solution RL	10 ml	160 ml	3x 160 ml
Binding Solution RBS	10 ml	125 ml	2x 250 ml
Washing Solution HS	5 ml (final volume 10 ml)	70 ml (final vol. 140 ml)	3 x 70 ml (final vol. 3x 140 ml)
Washing Solution LS	6 ml (final volume 30 ml)	2 x 50 ml (final vol. 2x 250 ml)	2 x 140 ml (final vol. 2x 700 ml)
RNase-free water	2 ml	2x 25 ml	4x 25 ml
Elution Tubes (1.5 ml)	15	5x 50	15x 50
KingFisher Tip Combs	3	50	150
KingFisher Tube Strips	15	250	750
Manual	1	1	1

	Σ 15	Σ 250	Σ 750
REF	845-KF-4515015	845-KF-4515250	845-KF-4515750
Initial steps	Washing Solution HS: • Add 5 ml of 96 – 99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! Washing Solution LS: • Add 24 ml of 96 – 99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed! Carrier Mix: • Add 1.25 ml RNase-free Water to the tube Carrier Mix, mix thoroughly by pipetting up and down!	Washing Solution HS: • Add 70 ml of 96 – 99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! Washing Solution LS: • Add 200 ml of 96 – 99.8 % ethanol to each bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed! Carrier Mix: • Add 1.25 ml RNase-free Water to each tube Carrier Mix, mix thoroughly by pipetting up and down!	Washing Solution HS: • Add 70 ml of 96 – 99.8 % ethanol to each bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! Washing Solution LS: • Add 560 ml of 96 – 99.8 % ethanol to each bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed! Carrier Mix: • Add 1.25 ml RNase-free Water to each tube Carrier Mix, mix thoroughly by pipetting up and down!

7 Recommended steps before starting

- Ensure that the Washing Solution HS and Washing Solution LS have been prepared according to the instruction (→ "Kit components" p. 7)
- Ensure that the Carrier Mix and Lysis Solution RL / Carrier Mix have been prepared according to the instruction (→ "Carrier Mix" p. 9)
- Centrifugation steps should be carried out at room temperature
- Avoid freezing and thawing of starting material

8 Components not included in the kit

- 1.5 ml reaction tubes
- 96 – 99.8 % ethanol

  Note: Use only absolute/pure ethanol, NO methylated or denatured alcohol!
- Buffer: PBS, optional for isolation of viral RNA from stool samples
- Physiological saline, optional (0.9 % NaCl for Influenca A testing)

9 Carrier Mix

9.1 Storage conditions and handling

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

- Add dissolved Carrier Mix to Lysis Solution RL immediately
- Unused Carrier Mix should kept frozen at -20 °C
- Do not freeze and thaw the Carrier Mix more than 3 times
- Mixture of Lysis Solution RL and Carrier Mix is stable for 7 days at 4 °C
- Internal control RNA or DNA can be detected by real-time PCR using the corresponding assays, as shown below:

Name	Amount	Order-no.
Detection of IC DNA innuDETECT Internal Control DNA Assay	100 rxn	845-ID-0006100
Detection of IC RNA innuDETECT Internal Control RNA Assay	100 rxn	845-ID-0007100
Detection of IC DNA and IC RNA innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

9.2 Preparation of Lysis Solution RL / Carrier Mix

1. Add 1.25 ml RNase-free Water to each tube Carrier Mix.
2. Mix thoroughly by pipetting up and down!
3. After the preparation of Carrier Mix stock solution prepare the mixture of Lysis Solution RL/Carrier Mix as described in the following table:

Reagent	8 samples	48 samples	96 samples	n x samples
Lysis Solution RL	3 ml	18 ml	36 ml	360 µl x sample
Carrier Mix	100 µl	600 µl	1.2 ml	12 µl x sample

Note: If customized Extraction Controls should be used, please add these components to the Lysis Solution RL / Carrier Mix!

10 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 have to 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 ddH_2O.
- 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.

11 Lysis protocols for isolation of viral RNA from different starting materials

> **Note**
> Please add Carrier Mix to the initial lysis reaction (Lysis Solution RL + Carrier Nucleic Acid and/or Extraction Control).
> Please refer also to chapter "Carrier Mix" on page 9.

11.1 Protocol 1: Isolation of viral RNA from cell-free body fluids (serum, plasma, cerebrospinal fluid, liquor)

1. Transfer **300 µl Lysis Solution RL/Carrier Mix** into a 1.5 ml Tube and add **200 µl** of the **sample**.
2. Close the cap and vortex the 1.5 ml Tube for 10 sec.
3. Place the 1.5 ml Tube into a thermal mixer and incubate under continuous shaking for 15 minutes at room temperature.

   Note: Alternative the 1.5 ml Tube can be mixed by vortexing during the lysis (each 5 min for 5 sec).

11.2 Protocol 2: Isolation of viral RNA from cell culture supernatants

1. Transfer **300 µl Lysis Solution RL/Carrier Mix** into a1.5 ml Tube and add **200 µl** of the **cell culture supernatant** (cell culture medium).
1. Close the cap and vortex the 1.5 ml Tube for 10 sec.
2. Place the 1.5 ml Tube into a thermal mixer and incubate under continuous shaking for 15 minutes at room temperature.

   Note: Alternative the 1.5 ml Tube can be mixed by vortexing during the lysis (each 5 min for 5 sec).

11.3 Protocol 3: Isolation of viral RNA from swab samples

1. Place the swab into a 1.5 ml reaction tube and add **500 µl Lysis Solution RL/Carrier Mix.**

   Important Note: To get maximum yield of viral nucleic acids it is essential to leave the swab in the reaction tube during the complete lysis time. It is possible to cut the shaft of the swab, so that you can close the cap of the reaction tube. It is also possible to perform the lysis step with opened cap. The removal of the swab from the reaction tube ahead of time will lead to a dramatically reduced final yield!

Vortex shortly!

2. Place the reaction tube into a thermal mixer and incubate under continuous shaking for 15 minutes at room temperature.

   Note: Alternative the reaction tube can be mixed by vortexing during the lysis (each 5 min for 5 sec).

3. After lysis time carefully squeeze out the swab on the wall of the tube and discard the swab.

11.4 Protocol 4: Isolation of viral RNA from tissue biopsies

1. Transfer **500 µl Lysis Solution RL/Carrier Mix** into a 1.5 ml reaction tube and add about **1 - 5 mg** of the **tissue biopsy**
2. Close the cap and vortex the reaction tube for 10 sec.
3. Place the reaction tube into a thermal mixer and incubate under continuous shaking for 30 minutes at room temperature. Lysis time can be increased up to lysis of starting material is complete (60 min).

   Note: Alternative the reaction tube can be mixed by vortexing during the lysis (each 5 min for 5 sec). A longer lysis time can lead to a reduced yield and quality of some viral RNA's

4. After lysis centrifuge the reaction tube at max. speed for 1 minute to spin down unlysed material and follow the manual exactly for the next steps.

11.5 Protocol 5: Isolation of viral RNA stool samples (tested for Norovirus extraction)

Alternative 1: Solid stool sample:

1. Transfer about **0.05 – 0.1 g** of the **stool sample** into a 1.5 ml reaction tube and add **200 µl PBS** (not included in scope of delivery).
2. Vortex the sample for 5 sec and centrifuge it at max. speed for 3 min.
3. Transfer respectively **300 µl Lysis Solution RL/Carrier Mix** into an reaction tube and add the **clearified supernatant** of the stool sample from step 2.
4. Close the cap and vortex the reaction tube for 10 sec.

5. Place the reaction tube into a thermal mixer and incubate under continuous shaking for 15 minutes at room temperature.

   Note: Alternative the reaction tube can be mixed by vortexing during the lysis (each 5 min for 5 sec).

Alternative 2. Homogenized stool sample:

1. Centrifuge the homogenized stool sample to spin down still remaining particles.
2. Transfer **300 µl Lysis Solution RL/Carrier Mix** into an reaction tube and add the **200 µl** of the **clearified supernatant** of the stool sample from step 1.
3. Close the cap and vortex the reaction tube for 10 sec.
4. Place the reaction tube into a thermal mixer and incubate under continuous shaking for 15 minutes at room temperature.

   Note: Alternative the reaction tube can be mixed by vortexing during the lysis (each 5 min for 5 sec).

12 Preliminary steps of the KingFisher ml

12.1 Buffers

Note
During sample lysis pre-fill the tubes of the KingFisher Tube Strips with the following buffers respectively.

Tube A: 450 µl Binding Solution RBS
50 µl MAG Suspension

Note!
It is important to mix the MAG Suspension by vigorous shaking or vortexing before use (approx. 30 sec)!

Apply lysed sample to the Tube A of the KingFisher Tube Strip

Tube B: 500 µl Washing Solution HS

Tube C: 800 µl Washing Solution LS

Tube D: 800 µl Washing Solution LS

Tube E: 120 µl RNase-free water

12.2 Sample

Note
The following step will be done after the sample lysis!

After lysis (in case of the biopsy sample after centrifugation) transfer the lysed sample (**about 500 µl**) into the Tube A of the KingFisher Tube Strip. The final volume has to be 1 ml (lysed sample + Binding Solution RBS + MAG Suspension).

13 Automatic processing of the KingFisher ml

1. Place the filled KingFisher Tube Strips into the KingFisher system on the right position!
2. Place the KingFisher Tip Combs onto the magnetic track!
3. Start the program **"INNU_ViralRNA_KFml"!**

Note: If you use a disc, load the program "INNU_ViralRNA_KFml"!

Note

1. After finishing the extraction protocol, the Tube E of the Tube Strip contains the extracted RNA. Store the RNA under adequate conditions. We recommend to store the extracted RNA at –80 °C.

2. If the RNA contains carryover of magnetic particles, transfer the RNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 minute and pipette the supernatant with RNA into a new tube.

14 Related Products

Name	Amount	Order No.
Detection of IC DNA		
innuDETECT Internal Control DNA Assay	100 rxn	845-ID-0006100
Detection of IC RNA		
innuDETECT Internal Control RNA Assay	100 rxn	845-ID-0007100
Detection of IC DNA and IC RNA		
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100
Products for Reverse Transcription/qPCR		
innuSCRIPT Reverse Transcriptase [25 U/µl]	50 rxn (1.250 U)	845-RT-5000050
	200 rxn (5.000 U)	845-RT-5000200
innuSCRIPT One Step RT-PCR SyGreen Kit	100 rxn	845-RT-6000100
	200 rxn	845-RT-6000200
innuSCRIPT One Step RT-PCR Probe Kit	100 rxn	845-RT-7000100
	200 rxn	845-RT-7000200
Products for PCR & Gel Electrophoresis		
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
innuTaq RED DNA Polymerase (1 U/µl)	500 U	845-EZ-2000500
innuTaq Hot-A DNA Polymerase (5 U/µl)	500 U	845-EZ-3000500
innuTaq UltraPure DNA Polymerase (5 U/µl)	500 U	845-EZ-6000500
50x inNucleotide Mix (12,5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
25 mM $MgCl_2$ - Solution	3x 1.5 ml	845-AS-1000015
50 mM $MgCl_2$ - Solution	3x 1.5 ml	845-AS-1010015
PCR-grade H_2O	2.0 ml	845-AS-1800002
	5x 2.0 ml	845-AS-1800010
innuMIX rapidPCR MasterMix	100 rxn	845-AS-1600100
	200 rxn	845-AS-1600200
innuMIX Standard PCR MasterMix	100 rxn	845-AS-1700100
	200 rxn	845-AS-1700200
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200

Name	Amount	Order No.
innuSTAR 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3x 1.0 ml	845-ST-3010003
	6x 1.0 ml	845-ST-3010006
6x Loading Dye Orange G	3x 1.0 ml	845-ST-4010003
	6x 1.0 ml	845-ST-4010006
Products for qPCR		
innuMIX qPCR MasterMix Probe	100 rxn	845-AS-1200100
	200 rxn	845-AS-1200200
innuMIX qPCR MasterMix SyGreen	100 rxn	845-AS-1300100
	200 rxn	845-AS-1300200

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