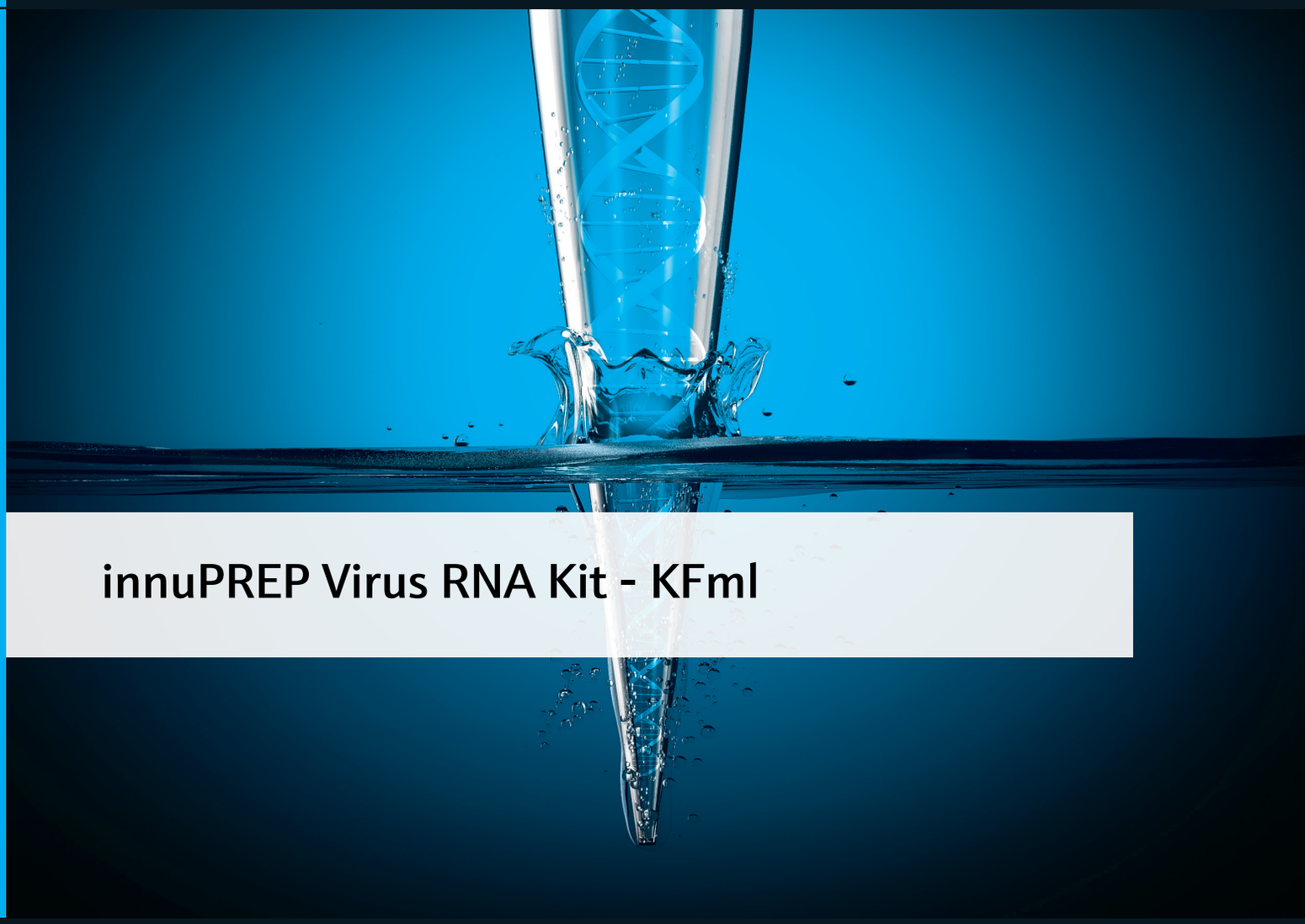
Instructions for Use
Life Science Kits & Assays



innuPREP Virus RNA Kit - KFml

Innuscreen GmbH

Order No.:
845-KF-4515015 15 reactions
845-KF-4515250 250 reactions
845-KF-4515750 750 reactions

Publication No.: HB_KF-4515_e_220228

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

Manufacturer and Distributor:
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!
Phone +49 30 9489 3380
Fax +49 30 9489 3381
info.innu@ist-ag.com

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.

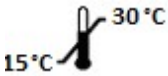
The extraction procedure is based on a new kind of chemistry. The procedure combines lysis of starting material with subsequent binding of viral RNA on surface modified magnetic particles. After washing steps, the viral RNA is eluted from the magnetic particles with water.

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

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. 3).
- 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 personnel 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. This kit could be used with potentially infectious samples. Therefore, all liquid waste must be considered 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 and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 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 hours or more before use. Autoclaving will not inactivate RNase activity 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 followed by autoclaving or heating to 100 °C for 15 minutes 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 water.
- 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.

4 Storage conditions

All kit components are shipped at ambient temperature.

Store lyophilized **Carrier Mix** at -22 °C to -18 °C. Divide dissolved **Carrier Mix** into aliquots and store at -22 °C to -18 °C. Do not freeze and thaw the **Carrier Mix** more than 3 times.

Store **MAG Suspension** at 4 °C to 8 °C.

The mixture of **Lysis Solution RL** and **Carrier Mix** is stable for a maximum of 7 days if stored at 4 °C to 8 °C.

All other components of the innuPREP Virus RNA Kit - KFml 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 dissolve these precipitates by careful warming. Before every use make sure that all components have room temperature.

5 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications 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 RNA Kit - KFml or other IST Innuscreen GmbH 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.

6 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. 9). 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 equivalents 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!

7 Kit components

7.1 Components included in the kit

	Σ 15	Σ 250	Σ 750
REF	845-KF-4515015	845-KF-4515250	845-KF-4515750
MAG Suspension	1.0 ml	2 x 9 ml	5 x 9 ml
Carrier Mix	1 x lyophilized powder	3 x lyophilized powder	7 x lyophilized powder
RNase-free water	1 x 2 ml	3 x 2 ml	7 x 2 ml
Lysis Solution RL	10 ml	160 ml	3 x 160 ml
Binding Solution RBS	10 ml	125 ml	2 x 250 ml
Washing Solution HS (conc.)	5 ml	70 ml	3 x 70 ml
Washing Solution LS (conc.)	6 ml	2 x 50 ml	2 x 140 ml
RNase-free water	2 ml	2 x 25 ml	4 x 25 ml
Elution Tubes (1.5 ml)	15	5 x 50	15 x 50
KingFisher Tip Combs	3	50	150
KingFisher Tube Strips	15	250	750
Manual	1	1	1

7.2 Components not included in the kit

- 1.5 ml tubes
- 96 %–99.8 % ethanol (molecular biology grade, non-denatured)
- PBS, optional (for isolation of viral RNA from stool samples)
- physiological saline, optional (0.9 % NaCl for Influenza A testing)

8 Usage of Carrier Mix

Besides carrier RNA, the **Carrier Mix** contains an Internal Control DNA and Internal Control RNA (IC DNA and IC RNA). Both 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

If customized extraction controls are used, please add these components to the mixture of **Lysis Solution RL / Carrier Mix** (→ "Initial steps before starting" p. 10).

9 Product specifications

1. Starting material:

- Cell-free body fluids and cell culture supernatant (e.g., serum, plasma, cerebrospinal fluid, liquor)
- Swabs from nasopharyngeal samples (e.g., Influenza testing)
- Tissue samples (up to 5 mg)
- Stool samples (50–100 mg)

2. Time for isolation:

- Approximately 35 minutes

10 Initial steps before starting

- Add the indicated amount of absolute ethanol to **Washing Solution HS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-KF-4515015	Add 5 ml ethanol to 5 ml Washing Solution HS (conc.)
845-KF-4515250	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.)
845-KF-4515750	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.)

- Add the indicated amount of absolute ethanol to **Washing Solution LS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-KF-4515015	Add 24 ml ethanol to 6 ml Washing Solution LS (conc.)
845-KF-4515250	Add 200 ml ethanol to 50 ml Washing Solution LS (conc.)
845-KF-4515750	Add 560 ml ethanol to 140 ml Washing Solution LS (conc.)

- Add 1.25 ml RNase-free water to each vial of **Carrier Mix**, mix thoroughly and store as described above.
- Prepare **Lysis Solution RL / Carrier Mix** according to the table below and store as described above.

Component	8 samples	48 samples	96 samples	n samples
Lysis Solution RL	3 ml	18 ml	36 ml	360 µl x n samples
Carrier Mix	0.1 ml	0.6 ml	1.2 ml	12 µl x n samples
Final volume	3.1 ml	18.6 ml	37.2 ml	372 µl x n samples

NOTE

If customized extraction controls are used, please add these components to the Lysis Solution RL / Carrier Mix!

- Avoid freezing and thawing of starting material.
- Centrifugation steps should be carried out at room temperature.

11 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 "Usage of 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 tube for 10 sec.
3. Place the tube into a thermal mixer and incubate under continuous shaking for 15 min. at room temperature.

NOTE

Alternatively, the sample can be mixed by vortexing during lysis (every 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).
2. Close the cap and vortex the tube for 10 sec.
3. Place the tube into a thermal mixer and incubate under continuous shaking for 15 min. at room temperature.

NOTE

Alternatively, the sample can be mixed by vortexing during lysis (every 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**.
   Vortex shortly.

NOTE

To obtain a 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 to 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!

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

NOTE

Alternatively, the sample can be mixed by vortexing during lysis (every 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 tube for 10 sec.
3. Place the tube into a thermal mixer and incubate under continuous shaking for 30 min. at room temperature. Lysis time can be increased until lysis of starting material is complete (60 min).

NOTE

Alternatively, the sample can be mixed by vortexing during lysis (every 5 min for 5 sec). A longer lysis time can lead to a reduced yield and quality of some viral RNAs.

4. After lysis, centrifuge the tube at max. speed for 1 minute to spin down unlysed material.

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

Option 1: Solid stool sample

1. Transfer about **50 – 100 mg** of the **stool sample** into a 1.5 ml reaction tube and add **200 µl PBS** (not included).
2. Vortex the sample for 5 sec. and centrifuge at max. speed for 3 min.
3. Transfer **300 µl Lysis Solution RL/Carrier Mix** into a new reaction tube and add the clarified supernatant of the stool sample from step 2.
4. Close the cap and vortex the tube for 10 sec.
5. Place the tube into a thermal mixer and incubate under continuous shaking for 15 min. at room temperature.

NOTE

Alternatively, the sample can be mixed by vortexing during lysis (every 5 min for 5 sec).

Option 2: Homogenized stool sample

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

NOTE

Alternatively, the sample can be mixed by vortexing during lysis (every 5 min for 5 sec).

12 Automated extraction using KingFisher ml

12.1 Pre-filling of King Fisher Tube Strips

NOTE

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

Fill the Tube Strips according to the table below:

Tube	Content
A	450 µl Binding Solution RBS + 50 µl MAG Suspension
B	500 µl Washing Solution HS
C	800 µl Washing Solution LS
D	800 µl Washing Solution LS
E	120 µl RNase-free water

12.2 Sample

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

12.3 Starting the automated extraction

1. Place the pre-filled KingFisher Tube Strips and Tip Combs into the KingFisher system into the right position and orientation.
2. Start the program **INNU_ViralRNA_KFml**.

NOTE

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

If the eluate contains carryover of magnetic particles, place the plate on a magnet or centrifuge the plate at maximum speed for 3 minutes. Pipet the supernatant with RNA into a new plate.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Poor lysis of starting material	
Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no total RNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
DNA contamination	
Too much starting material	Reduce amount of starting material.
Incorrect lysis of starting material	Use the recommended techniques for lysis of cell pellet.
Total RNA degraded	
RNA source inappropriately handled or stored	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, has been performed quickly.
RNase contamination of solutions; Receiver Tubes, etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream applications (e.g., RT-PCR)	
Salt carry-over during elution	Ensure that **Washing Solution HS** and **Washing Solution LS** are at room temperature. Check Washing Solution for salt precipitates. If there are any precipitates dissolve these by careful warming.

IST Innuscreen GmbH
Robert-Rössle-Str.10
13125 Berlin · Germany

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

IST Innuscreen GmbH