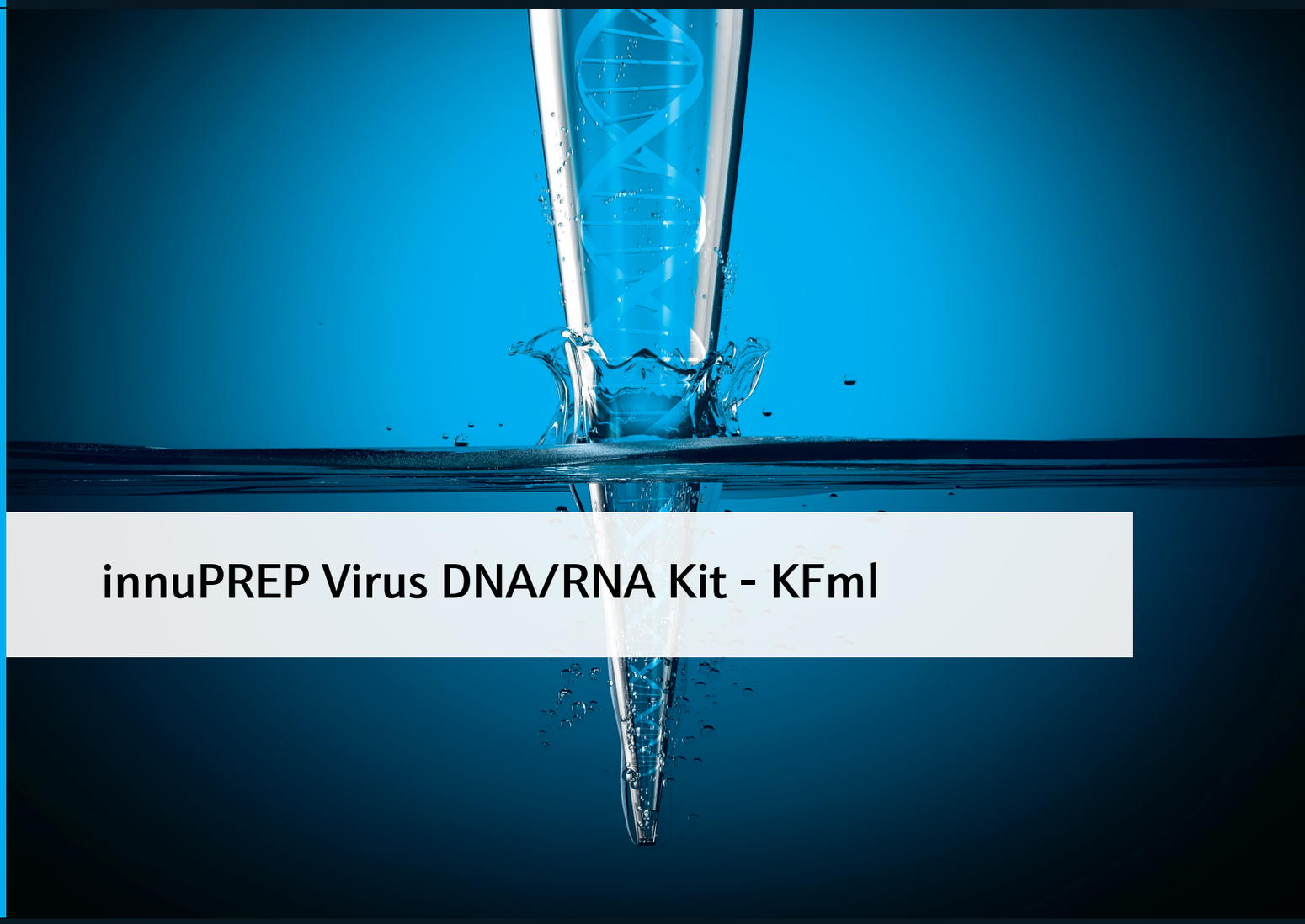
Instructions for Use
Life Science Kits & Assays



innuPREP Virus DNA/RNA Kit - KFml

Innuscreen GmbH

Order No.:

845-KF-4615015	15 reactions
845-KF-4615250	250 reactions
845-KF-4615750	750 reactions

Publication No.: HB_KF-4615_e_220322

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 DNA/RNA Kit - KFml has been designed for isolation of viral DNA/RNA from different kinds of starting material. The kit contains a Carrier Mix with Carrier RNA as well as an internal extraction controls 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 DNA/RNA on surface modified magnetic particles. After washing steps the viral DNA/RNA is eluted from the magnetic particles by using water.

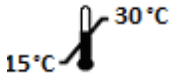
The extraction procedure takes place on the magnetic particle processor KingFisher ml. Extraction chemistry and extraction protocol are optimized to get maximum yield of RNA.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully 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> tests.
	**Storage conditions** Store at room temperature, unless otherwise specified.
	**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 device and to avoid operating errors for obtaining correct results.

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 is designed to 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 is to be used with potential infectious human samples. 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 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

The kit is 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 DNA/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 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 DNA/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 (→ “Intended use” p. 2) (→ “Product specifications” p. 10). 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 the 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-4615015	845-KF-4615250	845-KF-4615750
MAG Suspension	1 ml	2 x 9 ml	5x 9 ml
Carrier Mix	For 1 x 1.25 ml working solution	For 4 x 1.25 ml working solution	For 10 x 1.25 ml working solution
RNase-free Water	2 ml	4x 2 ml	10x 2 ml
Lysis Solution RL	10 ml	140 ml	2x 200 ml
Binding Solution RBS	10 ml	125 ml	2x 180 ml
Washing Solution HS (conc.)	5 ml	70 ml	3x 70 ml
Washing Solution LS (conc.)	6 ml	2x 50 ml	2x 130 ml
RNase-free Water	2 ml	2x 25 ml	4x 25 ml
KingFisher Tip Combs	3	50	150
KingFisher Tube Strips	15	250	750
Elution Tubes	15	5x 50	15x 50
Manual	1	1	1

7.2 Components not included in the kit

- 96–99.8 % ethanol, non-denatured or methylated 1.5 ml tubes
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- Physiological saline

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.11).

9 Product specifications

1. Starting material:
   - Cell-free body fluids (serum, plasma, cerebrospinal fluid, liquor); max. 200 µl sample volume
   - Cell culture supernatants; max. 200 µl sample volume
   - Swab samples ; max. 200 µl sample volume
   - Stool samples ; max. 200 µl sample volume
   - Tissue biopsies (1–5 mg)

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-4615015	Add 5 ml ethanol to 5 ml Washing Solution HS (conc.)
845-KF-4615250	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.)
845-KF-4615750	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-4615015	Add 24 ml ethanol to 6 ml Washing Solution LS (conc.)
845-KF-4615250	Add 200 ml ethanol to 50 ml Washing Solution LS (conc.)
845-KF-4615750	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	1 sample	5 samples	15 samples	n samples
Lysis Solution RL	0.5 ml	2.5 ml	7.5 ml	500 µl x n samples
Carrier Mix	16.5 µl	82.5 µl	247.5 µl	16.5 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 Lysis protocols for isolation of viral DNA/RNA from different starting materials

11.1 Protocol 1: Isolation of viral DNA and RNA from 200 µl 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 seconds.
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 minutes for 5 seconds).

11.2 Protocol 2: Isolation of viral DNA/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 1.5 ml Tube for 10 seconds.
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 minutes for 5 seconds).

11.3 Protocol 3: Isolation of viral DNA/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 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!

2. Place the reaction 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 minutes for 5 seconds).

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 DNA/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 seconds.
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 minutes).

NOTE
Alternative the reaction tube can be mixed by vortexing during the lysis (each 5 minutes for 5 seconds). A longer lysis time can lead to a reduced yield and quality of some viral DNA/RNA.

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 DNA/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 the kit).
2. Vortex the sample for 5 seconds and centrifuge it at max. speed for 3 minutes.
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 seconds.
5. Place the reaction 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 minutes for 5 seconds).

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 seconds.
4. Place the reaction 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 minutes for 5 seconds).

12 Preliminary steps of the KingFisher ml

12.1 Filling of KingFisher Tube Strips

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

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

Filling of KingFisher Tube Strips

Tube A	450 µl 50 µl	**Binding Solution RBS** **MAG Suspension**
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 Loading the 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** (approx. 500 µl) into the **Tube A** of the KingFisher Tube Strip.

The final volume has to be 1 ml (**lysed sample** and **Binding Solution RBS** and **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
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.

14 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted viral RNA/DNA	
Insufficient lysis of starting material.	Ensure to use the correct mixture of **Lysis Solution RL** and **Carrier Mix**. Ensure to incubate at room temperature for sample lysis.
Inadequate extraction.	Inhibiting substances in starting material. Please use the kit only for samples that match the requirements declared in “Product specifications”. Use Internal Controls for verification of extraction procedure.

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