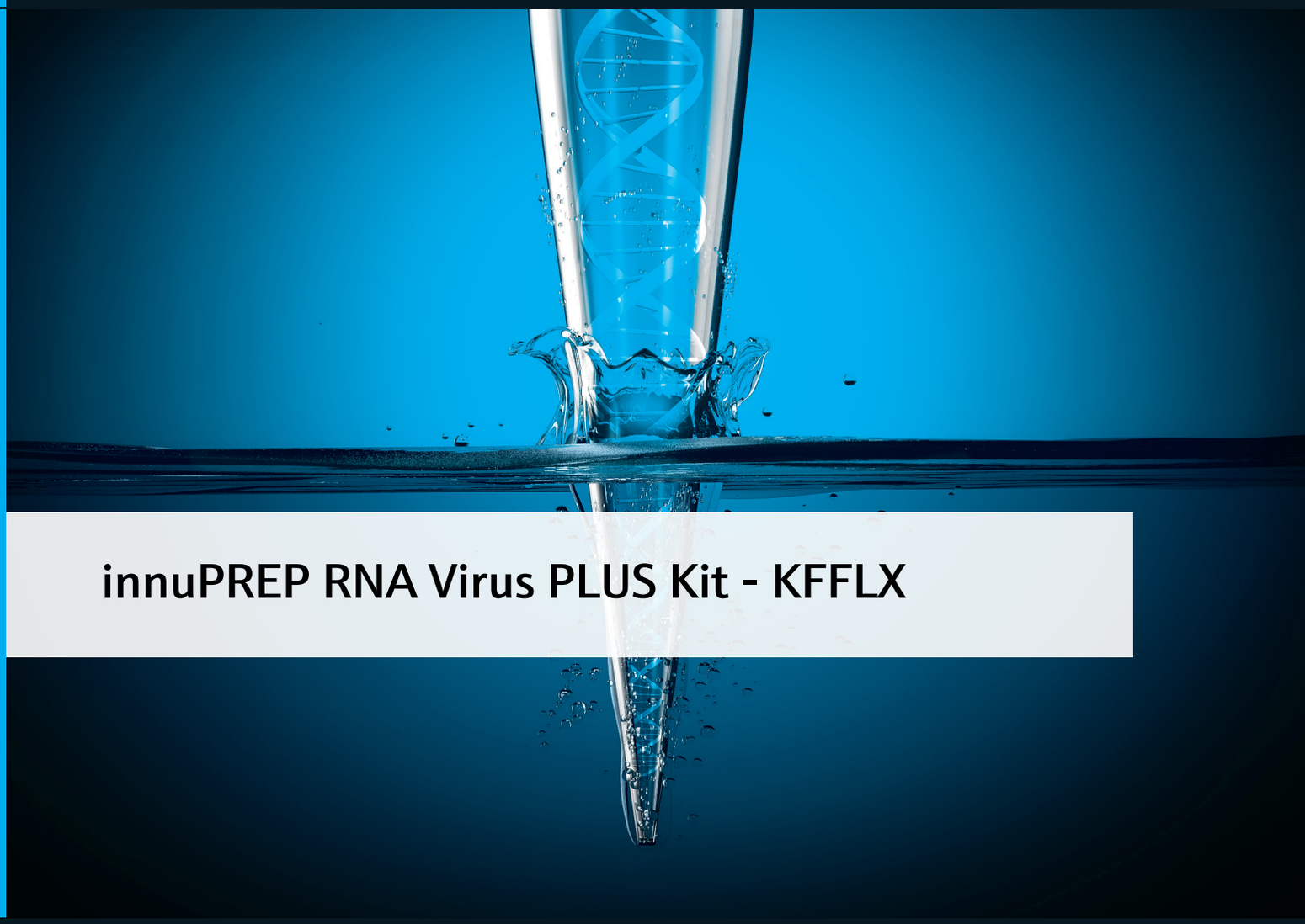
Instructions for Use
Life Science Kits & Assays



innuPREP RNA Virus PLUS Kit - KFFLX

Innuscreen GmbH

Order No.:
845-KF-5396096 96 reactions
845-KF-5396480 480 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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Introduction

1.1 Intended use

The innuPREP RNA Virus PLUS Kit - KFFLX has been designed for isolation of viral RNA from different kinds of starting material. The extraction procedure is based on a new kind of patented 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 by using water. The extraction process is running in two steps: The first step includes automated sample lysis and is followed by the second step which performs automated nucleic acid extraction.

The extraction procedure takes place on the magnetic particle processor KingFisher FLEX and allows the parallel extraction of up to 96 samples.

The kit contains a Carrier Mix. The Carrier Mix consists of a necessary Carrier RNA as well as a synthetic DNA fragment and MS2 RNA. Both can be used as internal extraction controls. The proof can be provided by means of available assays from IST Innuscreen GmbH. In addition, individual internal controls can be used. No data are available on the rate of recovery of individual used internal controls. There can be no guarantee for the recovery of individual internal controls. It also pointed out here that used individual controls based on MS2 RNA sequences may lead to higher detection signals with the MS2 RNA from the Carrier Mix.

It is important to note, that the kit should be used ever with an internal extraction control and corresponding detection assays to monitor the purification, amplification, and detection processes.

Please note that the eluates contain Carrier Mix. In case of using Carrier Mix the quantification of nucleic acids (isolated with this kit) by photometric or fluorometric methods is not possible. It is recommended to quantify extracted RNA/DNA with other methods like specific quantitative Real-time PCR.

The detection limit for certain viruses depends on the individual procedures, for example in-house PCR or commercial used detection assays. We can give no warranty for the efficiency of extraction for different kinds of viruses.

The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions and further analytical procedures. Diagnostic results generated using the extraction procedure in conjunction with diagnostic tests should be interpreted regarding other clinical or laboratory results. To reduce irregularities in diagnostic results, internal controls for downstream applications should be 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	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15 °C 30 °C	**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	**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 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 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

All kit components are shipped at ambient temperature.

Upon arrival store **MAG Suspension** at 4 °C to 8 °C and lyophilized **Carrier Mix** at -22 °C to -18 °C. Aliquot dissolved **Carrier Mix** and do not freeze and thaw it more than 3 times!

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 RNA Virus PLUS Kit - KFFLX 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.

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 kit 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 RNA Virus PLUS Kit - KFFLX 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). Since the performance characteristics of our kits have not been validated for any specific application. 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 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 Included kit components

	Σ 96	Σ 480
REF	845-KF-5396096	845-KF-5396480
MAG Suspension	**5.5 ml**	**3 x 9 ml**
Carrier Mix	for 1 x 1.25 ml working solution	for 5 x 1.25 ml working solution
RNase-free Water	2ml	5 x 2 ml
Lysis Solution RL	60 ml	2 x 150 ml
Binding Solution RBS	50 ml	250 ml
RNase-free Water	15 ml	3 x 25 ml
Washing Solution HS (conc.)	30 ml	2 x 70 ml
Washing Solution LS (conc.)	36 ml	180 ml
Washing Solution D	60 ml	250 ml
KF96 Tip Comb with DW Plate	1	5
KF96 Elution Plate	1	5
KF96 DW Plate	5	25
Manual	1	1

7.2 Components not included in the kit

- 1.5 ml tubes
- 96–99.8 % ethanol (non denaturated or methylated)
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4); optional for Protocol 5
- 0.9 % physiological saline (NaCl); optional for Protocol 3

8 Product specifications

1. Starting material:

- Cell-free body fluids and cell culture supernatant (e.g. serum, plasma, cerebrospinal fluid)
- Swabs from nasopharyngeal samples (e.g. Influenza A)
- Tissue samples (up to 10 mg)
- Stool samples (50–100 mg) (e.g. Norovirus)

2. Time for isolation:

- Approximately 60 minutes

9 Initial steps before starting

- Add the indicated amount of RNase-free Water to each vial **Carrier Mix**, mix thoroughly and store as described above.

845-KF-5396096 845-KF-5396480	Add 1.25 ml RNase-free Water to lyophilized Carrier Mix.

- 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-KF-5396096	Add 30 ml ethanol to 30 ml Washing Solution HS (conc.).
845-KF-5396480	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

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

845-KF-5396096	Add 144 ml ethanol to 36 ml Washing Solution LS (conc.).
845-KF-5396480	Add 720 ml ethanol to 180 ml Washing Solution LS (conc.).

- Centrifugation steps should be carried out at room temperature.

- Prepare Lysis Solution RL / Carrier Mix as described (→ p. 11).

10 Usage of Carrier Mix

10.1 Detection

Besides carrier RNA, the **Carrier Mix** contains an Internal Control DNA and RNA (IC DNA and IC RNA). Both can be detected by real-time PCR using the corresponding assays.

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

10.2 Preparation of Lysis Solution RL / Carrier Mix

Prepare mixture of **Lysis Solution RL** and **Carrier Mix** according to the table below.

Component	8 samples	48 samples	96 samples	n 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
Final volume	3.1 ml	18.6 ml	37.2 ml	372 µl x sample

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

NOTE
Store mixture of **Lysis Solution RL / Carrier Mix** at 4–8 °C for a maximum of 7 days.

11 Automated sample lysis for different starting materials

IMPORTANT NOTE

The extraction protocol is based on two automated runs:

Step I: Sample lysis on KingFisher FLEX

Step II: Extraction of viral RNA on KingFisher FLEX

11.1 Protocol 1: Isolation from cell-free body fluids

1. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells. Add **200 µl** of the **sample** to each well containing Lysis Solution RL/Carrier Mix.
2. Follow chapter 11.6 “Settings of KingFisher FLEX for automated lysis” on page 16.

11.2 Protocol 2: Isolation from cell culture supernatants

1. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells. Add **200 µl** of the **cell culture supernatant (cell culture medium)** to each well containing Lysis Solution RL/Carrier Mix.
2. Follow chapter Follow chapter 11.6 “Settings of KingFisher FLEX for automated lysis” on page 16.

11.3 Protocol 3: Isolation from swab samples

Alternative 1:

1. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells.
2. Place the swabs into 1.5 ml reaction tubes containing **500 µl physiological saline** (0.9 % NaCl), incubate short and shake the swab vigorously inside the solution, squeeze it at the wall of the tube and remove the swab.

3. Transfer **200 µl** of the **physiological saline** to each well of the "Binding Plate" containing **Lysis Solution RL/Carrier Mix**.

4. Follow chapter 11.6 "Settings of KingFisher FLEX for automated lysis" on page 16

Alternative 2:

1. Transfer **550 µl Lysis Solution RL/Carrier Mix** into the 1.5 ml reaction tubes.

2. Place the swabs into the 1.5 ml reaction tubes containing Lysis Solution RL/Carrier Mix, incubate short and shake the swab vigorously inside the solution, squeeze it at the wall of the tube and remove the swab.

3. Label one 96 DW Plate (Deep Well Plate) with "Binding Plate" and transfer **approx. 500 µl** of the **sample** into the wells.

4. Follow chapter 11.6 "Settings of KingFisher FLEX for automated lysis" on page 16.

11.4 Protocol 4: Isolation from tissue biopsies

Alternative 1:

1. Transfer **500 µl Lysis Solution RL/Carrier Mix** into a 1.5 ml reaction tube and add approx. **1 – 5 mg** of the **tissue biopsy**.

2. Close the cap and vortex the 1.5 ml reaction tube for 10 sec.

3. Place the 1.5 ml 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 1.5 ml 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 1.5 ml reaction tube at max. speed for 1 minute to spin down unlysed material.

5. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer approx. **500 µl of the lysed sample** into the wells.

6. Follow chapter 11.6 “Settings of KingFisher FLEX for automated lysis” on page 16.

Alternative 2:

1. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer approx. 300 µl Lysis Solution RL/Carrier Mix into the wells.

2. Transfer **300 µl Lysis Solution RL/Carrier Mix** into a 1.5 ml reaction tube and add approx. **10 – 20 mg** of the **tissue biopsy**.

3. Close the cap and vortex the 1.5 ml reaction tube for 10 sec.

4. Place the 1.5 ml 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).

5. Note: Alternative the 1.5 ml 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

6. After lysis centrifuge the 1.5 ml reaction tube at max. speed for 1 minute to spin down unlysed material.

7. Transfer **200 µl of the lysed sample** to each well of the “Binding Plate” containing Lysis Solution RL/Carrier Mix.

8. Follow chapter 11.6 “Settings of KingFisher FLEX for automated lysis” on page 16.

11.5 Protocol 5: Isolation of viral RNA from stool samples

Alternative 1:

1. Transfer about **0.05 – 0.1 g** of the **stool sample** into a 1.5 ml reaction tube and add **250 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. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells and add the **clarified supernatant (max. 250 µl)** of the stool sample from step 2 to each well of the “Binding Plate” containing Lysis Solution RL/Carrier Mix.
4. Follow chapter 11.6 “Settings of KingFisher FLEX for automated lysis” on page 16.

Alternative 2.

In some cases, the initial fecal sample is mixed with special ELISA Buffer for subsequent ELISA detection of Norovirus.

1. Transfer **250 µl of the sample** into a 1.5 ml reaction tube and centrifuge the tube at maximum speed for 3 minutes.
2. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells and add the **clarified supernatant (max. 250 µl)** of the stool sample from step 1 to each well of the “Binding Plate” containing Lysis Solution RL/Carrier Mix.
3. Follow chapter 11.6 “Settings of KingFisher FLEX for automated lysis” on page 16.

11.6 Settings of KingFisher FLEX for automated lysis

1. Label the KF 96 Tip Comb with 96 DW Plate with "Tip Comb"
2. Switch on KingFisher FLEX and select the protocol "RNA SAMPLE LYSIS"
3. Follow the instructions shown on the display and load the Tip Comb and 96 DW Plate successively and start the automated lysis

NOTE
After sample lysis protocol plate "Tip Comb" and "Binding Plate" will further be used.

12 Automated extraction of viral ribonucleic acids

12.1 Prefilling of Deep Well Plates and Elution Plate

NOTE
During sample lysis label the DW Plates and pre-fill all needed buffers into the wells of the DW Plates and the 96 Plate as described below!

1. After lysis protocol remove the “Binding Plate” and the plate “Tip Comb” from the KingFisher FLEX.
2. Add Add **450 µl of Binding Solution RBS** and **50 µl MAG Suspension** to each well containing the sample and Lysis Solution RL/Carrier Mix.

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

3. Label and fill the Deep Well plates according to the following table.

KF96 DW Plate	Buffer
Washing Plate 1	500 µl Washing Solution HS
Washing Plate 2	800 µl Washing Solution LS
Washing Plate 3	800 µl Washing Solution LS
Washing Plate 4	500 µl Washing Solution D

4. Pre-fill the KF96 Elution Plate

KF96 Elution Plate	Buffer
Elution Plate	120 µl RNase-free water

12.2 Settings of King Fisher FLEX and automated extraction run

5. Switch on KingFisher Flex and select the protocol “INNU_VirusRNA_P_KFFLX”.

6. Follow the instructions shown on the display and load the Tip Comb, KF96 DW Plates and KF96 Elution Plate successively.
   - Tip Comb (re-used from Chapter 11)
   - Elution Plate
   - Washing Plate 4
   - Washing Plate 3
   - Washing Plate 2
   - Washing Plate 1
   - Binding Plate (containing lysed sample, Binding Solution RBS and MAG Suspension)
7. Start the automated extraction.

IMPORTANT NOTE

After finishing the extraction protocol, the Elution Plate contains the isolated DNA/RNA. Store the DNA/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 DNA/RNA into a new plate.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Poor lysis of starting material	
Insufficient disruption or homogenization	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 carryover during elution	Ensure that **Washing Solution HS** and **Washing Solution LS** are at room temperature. Checkup Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.

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