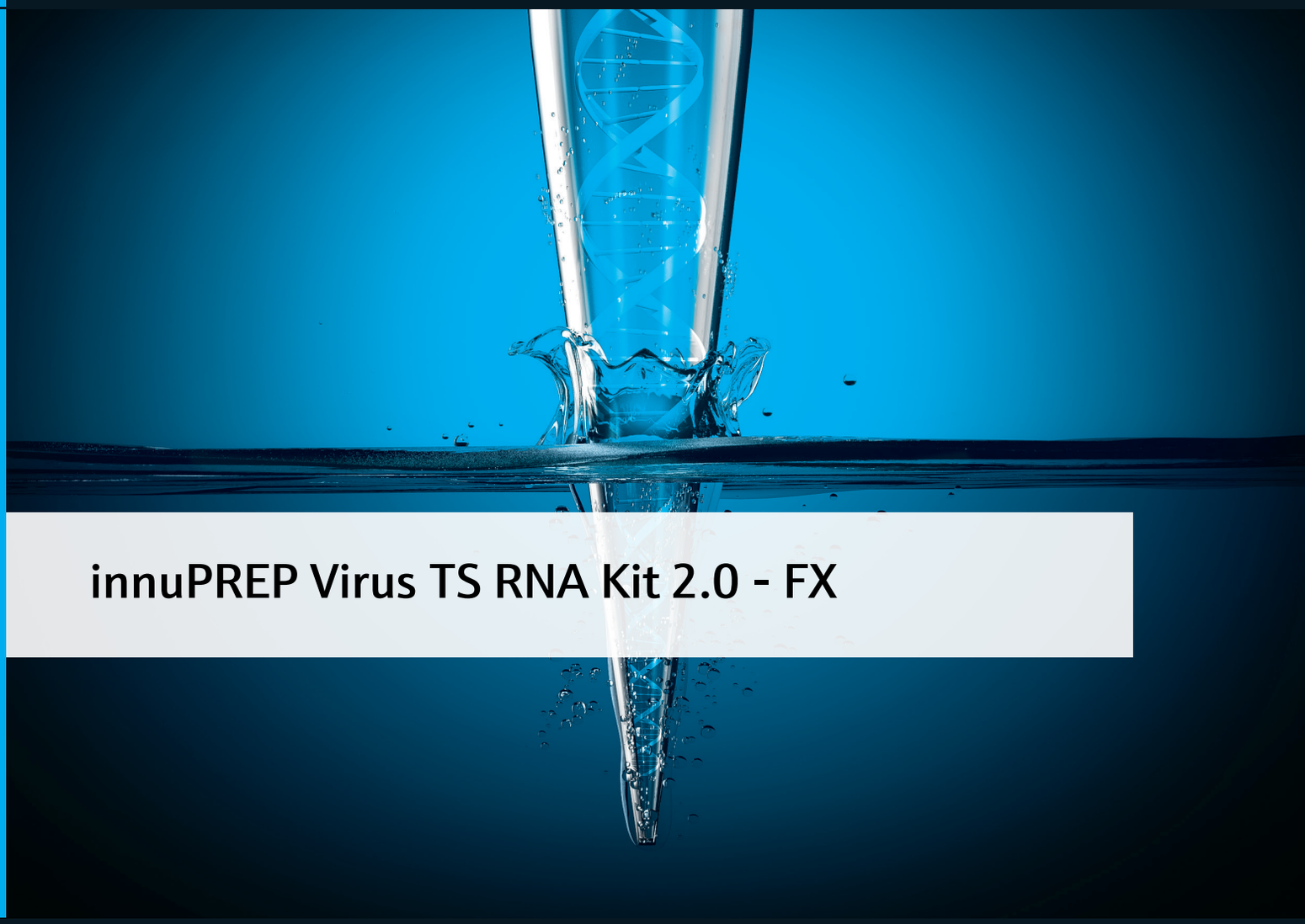
Instructions for Use
Life Science Kits & Assays



innuPREP Virus TS RNA Kit 2.0 - FX

Innuscreen GmbH

Order No.:

845-FX-2796096 96 reactions
844-27969600 9600 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!

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

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1 Introduction

1.1 Intended use

The **innuPREP Virus TS RNA Kit 2.0 – FX** has been designed for the completely automated isolation of viral RNA from tracheal swabs. The extraction procedure is based on a newly patented chemistry. The kit is designed to be handled by educated personnel in a laboratory environment.

All steps of the extraction process are automated and run completely on the CyBio FeliX. The extraction process is based on binding of RNA to surface-modified magnetic particles. After several washing steps, the nucleic acids are eluted from the magnetic particles with RNase-free Water and are ready to be used in downstream applications. The extraction chemistry in combination with the CyBio FeliX protocol is optimized to get maximum yield and quality.

Further, the kit contains a Carrier RNA for better recovery of minimal amounts of sample RNA.

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. Please note that the eluates of the kit contain both, sample nucleic acids and Carrier RNA. Therefore, it is not possible to quantify the isolated nucleic acids by photometric or fluorometric methods when using the Carrier RNA. Thus, other methods for quantification such as specific quantitative PCR or real-time PCR systems are recommended. Furthermore, Carrier RNA may inhibit PCR reactions. The amount of added Carrier RNA must therefore be carefully optimized depending on the individual PCR system used.

CONSULT INSTRUCTIONS 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 kit (labeling)

For easy reference and orientation, the manual uses 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> tests.
	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 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. 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.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling the 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!
Do not 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. IST Innuscreen GmbH 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 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.

Upon arrival, store **MAG Suspension** and lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

Store lyophilized and dissolved **Carrier RNA** at -22 °C to -18 °C. Aliquot dissolved **Carrier RNA** and do not freeze and thaw it more than 3 times!

All other components of the **innuPREP Virus TS RNA Kit 2.0 – FX** 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.

Before every use make sure that all components are at room temperature. If there are any precipitates within the provided solutions, they can be dissolved by careful warming.

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 was 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 2.0 – FX** or other IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information please contact info.innu@ist-ag.com or 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 this manual (→ “Product specifications”, p. 14). 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 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
For research use only!

7 Kit components

7.1 Included kit components

	Σ 96	Σ 9600	Required* per run (96 samples) *including surplus for automated prefilling
REF	845-FX-2796096	844-27969600	
MAG Suspension	9 mL	100 x 9 mL	9 mL
Proteinase K	for 1 x 1.5 mL working solution	For 5 x 30 mL working solution	1.5 mL working solution
Carrier RNA	for 1 x 1.0 mL working solution	for 100 x 1.0 mL working solution	1.0 mL working solution
Lysis Solution CBV	85 mL	8500 mL	85 mL
RNase-free Water (for Elution)	70 mL	- (1)	70 mL
Washing Solution LS (conc.)	25 mL	2 x 1800 mL For 2 x 9000 mL working solution	25 mL conc. For 125 mL working solution
Deep Well Plate (square, 2.0 mL)	7	700	7
Final Elution Plate (2.0 mL)	1	100	1
Protective Plate	2	200	2
Sealing Foil	1	100	1
Filter Tips	2 x 96	200 x 96	2 x 96
Manual	1	1	1

(1) Has to be provided by user

7.2 Components not included with the kit

- 1.5 mL and 15 mL tubes for swab incubation
- Physiological saline for swab incubation
- 96–99.8% Ethanol (molecular biology grade, undenatured) for dilution of Washing Solution LS (conc.)
- 80% Ethanol (molecular biology grade, undenatured) for washing steps
- 2-Propanol (molecular biology grade) for binding step
- RNase-free Water for dissolving Proteinase K and Carrier RNA
- Only for 844-27969600: RNase-free Water for Elution
- Pipetting tips for reagent prefilling
- 2 column and 12 column reservoirs for prefilling by CyBio FeliX (innuPREP Prefilling Set, OL3317-25-127)

7.3 Required CyBio FeliX components

- CyBio FeliX Basic Unit with Enclosure and CyBio Composer Software (OL5015-24-100, Analytik Jena GmbH)
- CyBio FeliX Extraction Set (OL5015-25-120) including Application Studio CyBio FeliX *eXtract* (Version 2.1.0.0 or higher)
- System-specific, pre-configured Laptop (820-90002-2, Analytik Jena GmbH)

7.4 Related products

- Protective Plate (OL3317-25-125, 50 pcs, Analytik Jena GmbH)
- Optical sealing foil (77 x 140 mm) (846-050-258-5D, 5 pcs, Analytik Jena GmbH)
- Filter Tips (OL3811-25-939-F, 16 x 96 pcs, Analytik Jena GmbH)
- Deep Well Plate (96 square well, 2.0 mL) (845-FX-8500025, 25 pcs, IST Innuscreen GmbH)
- Deep Well Plate (96 square well, 2.0 mL) (845-FX-8500115, 115 pcs, IST Innuscreen GmbH)
- Carrier RNA (for 1.0 mL ready-to-use solution) (31-00278, IST Innuscreen GmbH)
- innuDETECT Internal Control DNA/RNA Assay - 100 reactions à 25 µL (845-ID-0008100, IST Innuscreen GmbH)

NOTE
Only use disposable tips and plates included in recommended kits. The usage of other tips, reservoirs and plates may cause severe damage to the CyBio FeliX and a loss of warranty.
Also, the usage of other components may cause malfunction of the whole protocol and loss of samples!

8 Product specifications

1. Starting material:

   Swabs dry, delivered in physiological saline, liquid VTM, liquid amies und liquid UTM

2. Time for extraction:

Sample volume	Automated prefilling	Extraction	Elution volume
200 µL	49 min	70 min	50–200 µL

3. Typical yield

   Depends on amount, quality and infection progress of sample material. Avoid freezing and thawing of starting material.

9 Initial steps before starting

- Add the indicated amount of ddH_2O to each vial of lyophilized **Proteinase K**, mix thoroughly and store as described above.

845-FX-2796096	Add 1.5 mL ddH_2O to each vial of lyophilized **Proteinase K.**
844-27969600	Add 30 mL ddH_2O to each bottle of lyophilized **Proteinase K.**

- Add 1.0 mL RNase-free Water to each vial of lyophilized **Carrier RNA,** mix thoroughly and store as described above.
- Add absolute ethanol to **Washing Solution LS (conc.)** as noted in the table below and mix thoroughly. Keep the bottle always firmly closed!

845-FX-2796096	Add 100 mL ethanol to Washing Solution LS (conc.).
844-27969600	Add 7.200 mL ethanol to each can Washing Solution LS (conc.).

- Put accessories on corresponding supports according to the following table.

Accessories	Support
CyBio RoboTipTray 1-96/1000 µL (OL3810-13-023)	Support; 97 mm height (OL3317-11-105)
Gripper (OL3317-11-800)	Support; 37 mm height (OL3317-11-120)
8-channel adapter Head R (OL3317-14-330)	Support; 37 mm height (OL3317-11-120)
Cover Magazine Head R (OL30-3316-200-11)	Support; 37 mm height (OL3317-11-120)

See Figure 1 in order to differentiate between CyBio RoboTipTray 1-96/1000 µL and CyBio TipRack 96/1000 µL.

Figure 1: Difference between CyBio RoboTipTray 1-96/1000 µL (left) and TipRack 96/1000 µL (right).

NOTE

Please use the accessories only with the indicated supports! Usage of other supports or no supports may cause damage to the CyBio FeliX.

10 Prefilling of Reagent Plates

There is the option to prefill the plates automatically with the CyBio FeliX (→ see section 10.1) or manually (→ see section 10.2).

10.1 Automated prefilling with CyBio FeliX

NOTE
For the correct orientation of labware use position A1 marked on reservoirs and plates. The position A1 has to be on the top left corner of the CyBio FeliX deck (→ see Figure 2).

NOTE
Due to interactions of **MAG Suspension** with buffer components, the stability of magnetic particles in the Process Plate cannot be guaranteed. Therefore, the prefilling is only recommended when prefilled plates are used for the extraction process immediately after prefilling.

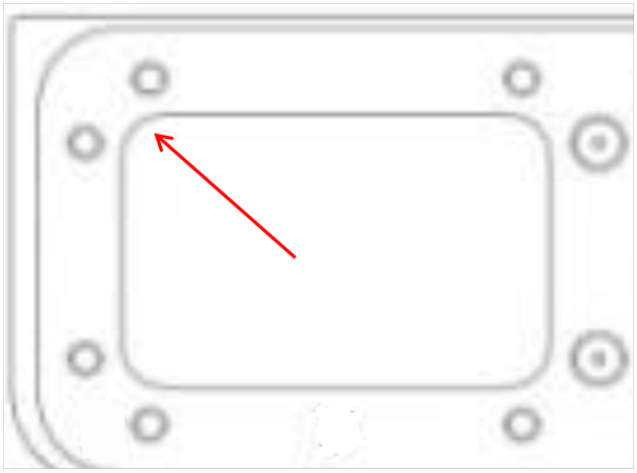

Figure 2: Positioning of plates and reservoirs on CyBio FeliX deck.

1. Label three reservoirs from the innuPREP Prefilling Set (→ see section 7.2 “Components not included”, p. 10) according to the table below:

Number	Label	
Reservoir 1 (2 column)	**Reservoir 1:** Left side of reservoir: Right side of reservoir:	 2-Propanol RNase-free Water (for Elution)
Reservoir 2 (2 column)	**Reservoir 2:** Left side of reservoir: Right side of reservoir:	 Washing Solution LS 80% Ethanol
Reservoir 3 (12 column)	**Reservoir 3:** Column 1	 MAG Suspension

2. Label the Deep Well Plates according to the following table:

Plate	Label
Plate 1*	Sample / Lysis
Plate 2	Process
Plate 3*	Waste (empty)
Plate 4	Washing Solution LS
Plate 5	80% Ethanol
Plate 6*	Elution (empty)
Plate 7	RNase-free Water (for Elution)
Plate 8*	Final Elution Plate (empty)

* Not required in the prefilling process, but for the extraction process. Put aside during prefilling.

3. Transfer 70 mL **2-Propanol** into the **left** side of the 2 column reservoir labeled “Reservoir 1 – 2-Propanol / RNase-free Water (for Elution)”.
4. Transfer the content of the bottle **“RNase-free Water (for Elution)”** (70 mL) into the **right** side of the 2 column reservoir labeled “Reservoir 1 – 2-Propanol / RNase-free Water (for Elution)”. Place the filled reservoir into the CyBio FeliX on position 2 (→ see Figure 3).
5. Transfer 125 mL of **“Washing Solution LS”** into the **left** side of the 2 column reservoir labeled “Reservoir 2 – Washing Solution LS / 80% Ethanol”.
6. Transfer 120 mL **80% Ethanol** into the **right** side of the 2 column reservoir labeled “Reservoir 2 – Washing Solution LS / 80% Ethanol”. Place the filled reservoir into the CyBio FeliX on position 5 (→ see Figure 3).
7. Vortex the 9 mL-bottle **MAG Suspension** properly (at least 30 s). Transfer the complete content of the bottle into column A1 of the reservoir labeled “Reservoir 3 – MAG Suspension”. Place the filled reservoir into the CyBio FeliX on position 12 (→ see Figure 3).
8. Insert Filter Tips in columns 1-7 in the CyBio TipRack 96/1000 µL. Please fill these columns completely with Filter Tips.
9. Place the CyBio TipRack 96/1000 µL into the CyBio FeliX on position 4 (→ see Figure 3).
10. Place the 8-channel adapter (Head R 96) with the support 37 mm into the CyBio FeliX on position 6 (→ see Figure 3).
11. Place the empty, labeled plates on the CyBio FeliX deck according to the deck layout for the prefilling protocol (→ see Figure 3).

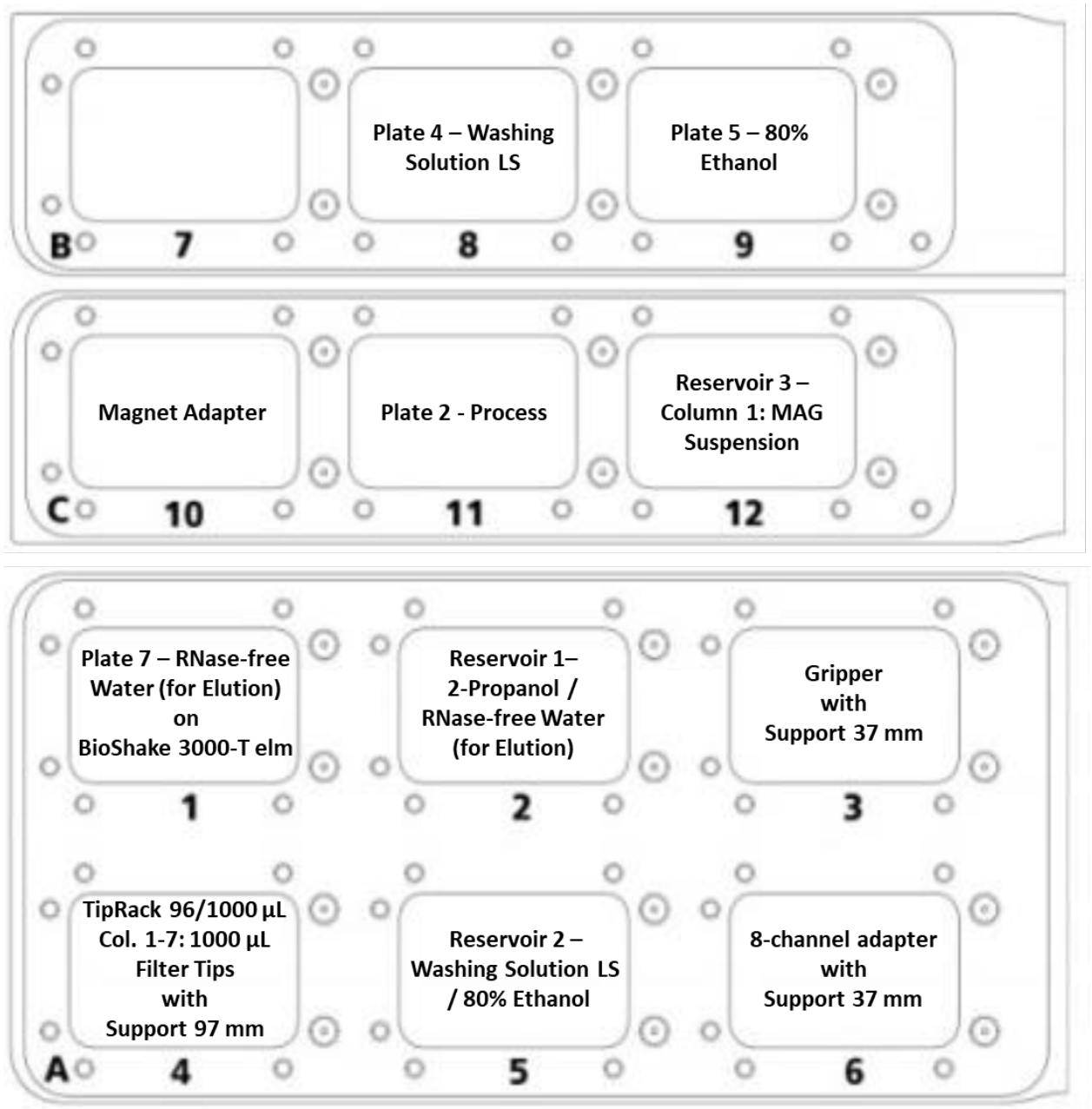

Figure 3: Deck layout for starting the prefilling protocol.

NOTE

Please pay special attention to the following deck position:

Position 1:

Place **Plate 7 – RNase-free Water (for Elution)** directly onto the BioShake 3000-T elm.

12. Switch on the CyBio FeliX and open the “AppStudio FeliX *eXtract*”.
13. Choose “Magnetic Beads” (→ see Figure 4).

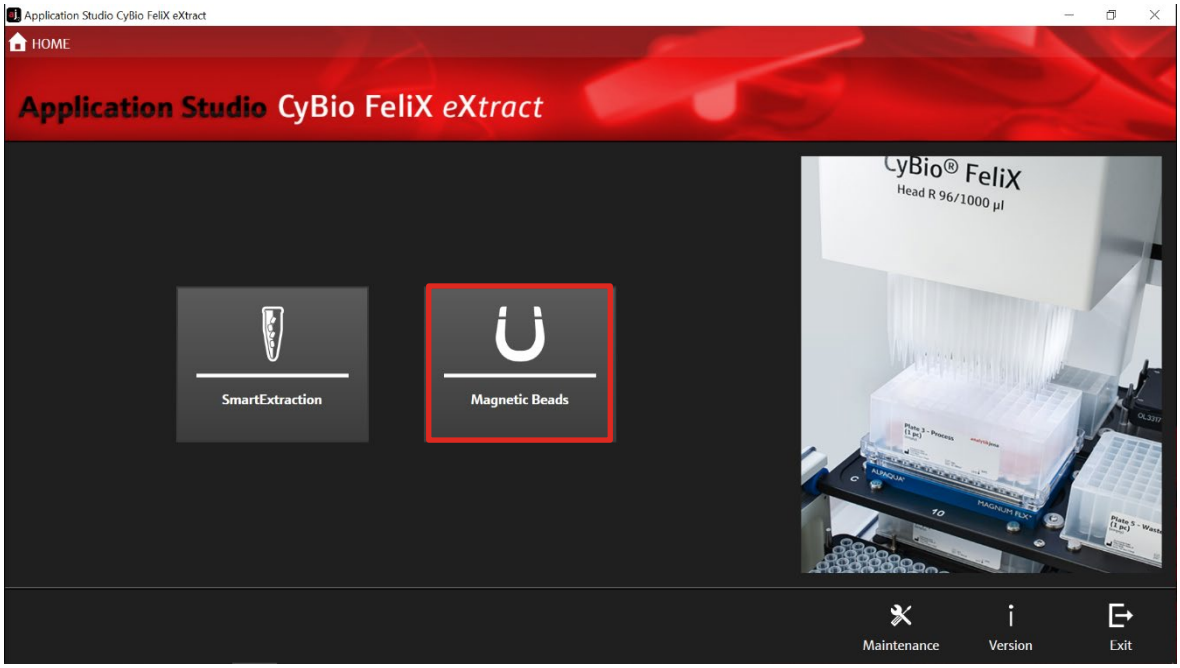

Figure 4: Homescreen of the AppStudio FeliX eXtract.

14. Choose “innuPREP Virus TS RNA Kit 2.0 – FX” (→ see Figure 5).

Figure 5: Kit selection in the AppStudio FeliX eXtract.

15. Choose “Prefilling” (→ see Figure 6).

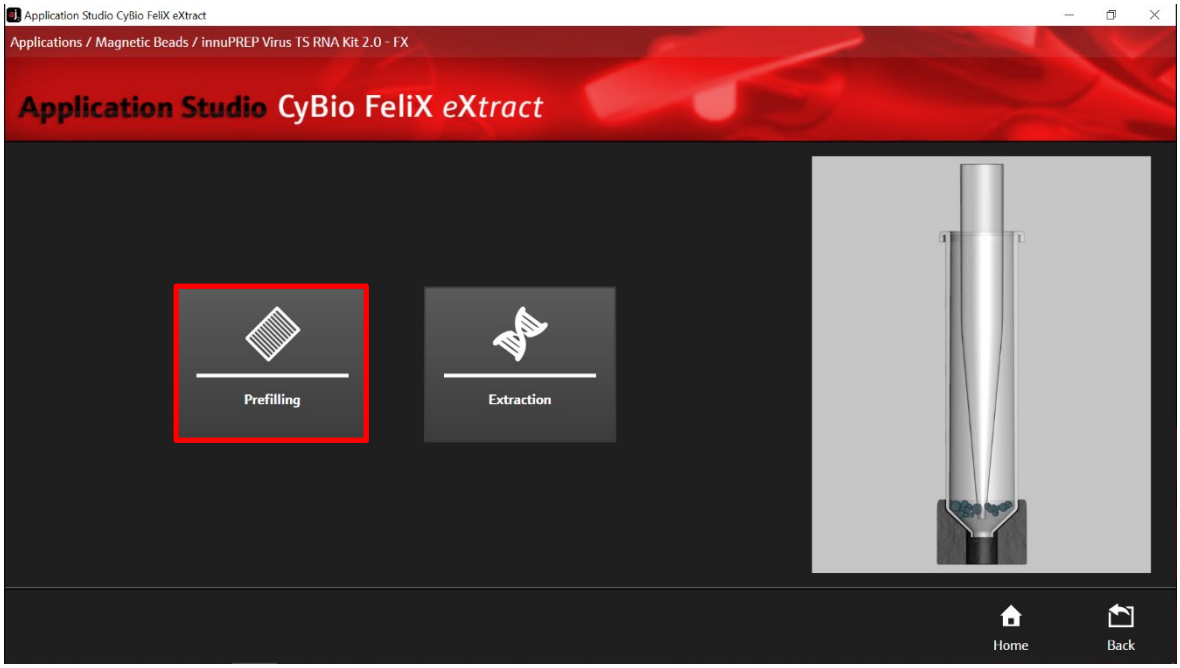

Figure 6: Routine selection in the AppStudio FeliX eXtract: Prefilling.

16. After choosing “Prefilling” the Prefilling Start Screen appears.
17. Check the correct version number of the protocol (→ see Figure 7): “Prefilling – innuPREP Virus TS RNA 2.0 – 02”.

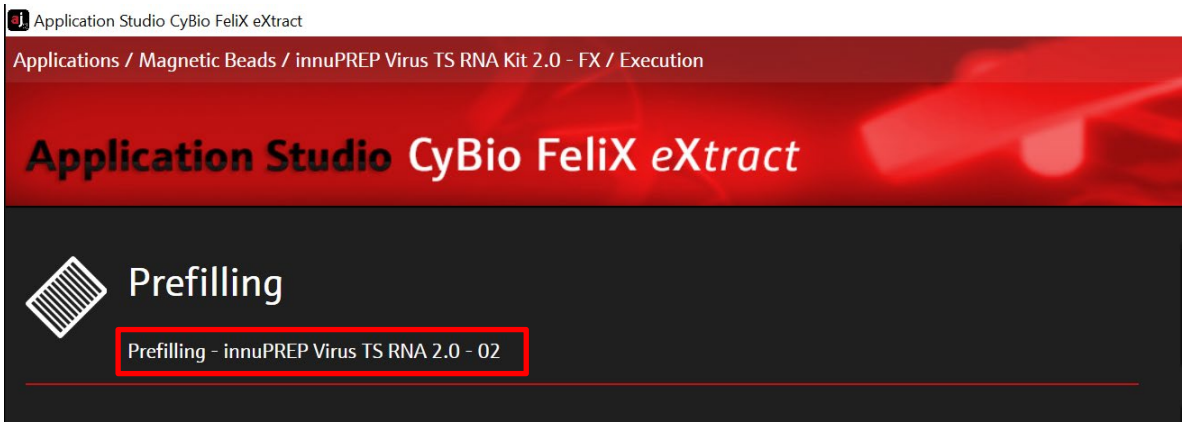

Figure 7: Version number of the prefilling protocol

18. Check the correct deck position of the plates, reservoirs and other hardware components (compare with list displayed in the AppStudio FeliX *eXtract*) and confirm with “Ok” (→ see Figure 8).

Figure 8: Deck layout for the final hardware check of the prefilling.

19. The chosen protocol is performed by the device. After the protocol is finished, the message “Prefilling completed” is shown on the screen of the computer. Confirm the message with “Ok” (→ see Figure 9).

Figure 10: Prefilling completed.

20. Remove the CyBio TipRack 96/1000 µL and discard all tips.
21. Remove 8-channel adapter (Head R 96) with Support 37 mm.
22. Discard the reservoirs and their contents.
23. The plates **Plate 4- Washing Solution LS**, **Plate 5 – 80% Ethanol**, **Plate 2 – Process** and the **Gripper with Support 37 mm** do not have to be removed for the extraction process.
24. **Plate 7 – RNase free Water (for Elution)** has to be removed from position 1 and placed on position 6.

NOTE
Please note that lysis buffer is not prefilled during the prefilling procedure. This kit is intended for RNA purification from swabs only and the lysis buffer must be added manually or used for direct swab incubation.

10.2 Manual prefilling

Please label and prepare the plates according to the table below.

Plate	Label	Content per well
Plate 1*	Sample / Lysis	empty
Plate 2	Process	**450 µL** 2-Propanol
Plate 3*	Waste	empty
Plate 4	Washing Solution LS	**1100 µL** Washing Solution LS
Plate 5	80% Ethanol	**1100 µL** 80% Ethanol
Plate 6*	Elution	empty
Plate 7	RNase-free Water (for Elution)	**600 µL** RNase-free Water (for Elution)
Plate 8*	Final Elution Plate	empty

* Not required in the prefilling process, but for the extraction process. Put aside during prefilling.

The deep well plates do not have to be filled completely. If less than 96 samples are to be extracted, only the required wells must be prefilled.

11 Protocol 1: Isolation from swabs stored in a storage solution

NOTE
To avoid mix-ups of samples, prepare a sample layout to assign the individual specimen to a well of the 96-well plate.

11.1 Preparing Process Plate & Sample / Lysis-Plate

NOTE
Step 1 does not have to be done when the prefilling is performed with the CyBio FeliX!

NOTE
Lysis of the sample material is done automatically and is included in the CyBio FeliX extraction protocol.

It is important to mix the **MAG Suspension** by vigorous shaking or vortexing before use (approx. 30 s). Repeated vortexing after 10 transfers is recommended.

1. Transfer **50 µL** of **MAG Suspension** directly into the liquid of each cavity of the prefilled plate **"Plate 2 – Process"**.
2. Transfer **200 µL** of the **Lysis Solution CBV** and **10 µL Carrier RNA** into **"Plate 1 – Sample / Lysis"**.
3. Open the storage tube with the storage medium. Shake the swab vigorously, squeeze it as completely as possible against the wall of the tube and remove the swab. Proceed with **200 µL of the particle-free sample** for further steps.
4. Add **200 µL** of the **sample** into the desired cavity of the prefilled plate **"Plate 1 – Sample / Lysis"**. Please adhere to your sample layout.
5. Add **10 µL Proteinase K** into the prefilled cavities of **"Plate 1 – Sample / Lysis"**.

11.2 Loading of the CyBio FeliX

1. Load all plates and accessories according to the scheme below (→ see Figure 10).
   As a Final Elution Plate (**Position 12**) multiple options are possible:
   - Plate 8 - Final Elution Plate
   - Micronic 750 µL pre-capped and racked 2D-tubes (MP52706-Y20)
   - Greiner Cryo.S 600 µL pre-racked (977561, 977580)

NOTE
Please pay special attention to the following deck positions:

Position 1: Place **Plate 1 – Sample / Lysis** on the BioShake 3000-T-elm (**deck position 1**).

Position 6: Stack **Plate 6 – Elution (empty)** directly on **Plate 7 – RNase-free Water (for Elution)**.

Position 2 and 5: Put the **Protective Plate** directly on the bottom plate of the **97 mm support**. Fill 96 Filter Tips (or the number of tips required) into the **CyBio RoboTipTray 1-96/1000 µL** using the **Tip Transfer Tool 96/1000 µL** and put it on the **97 mm support**. Take care that every Filter Tip fits into a cavity of the Protective Plate.

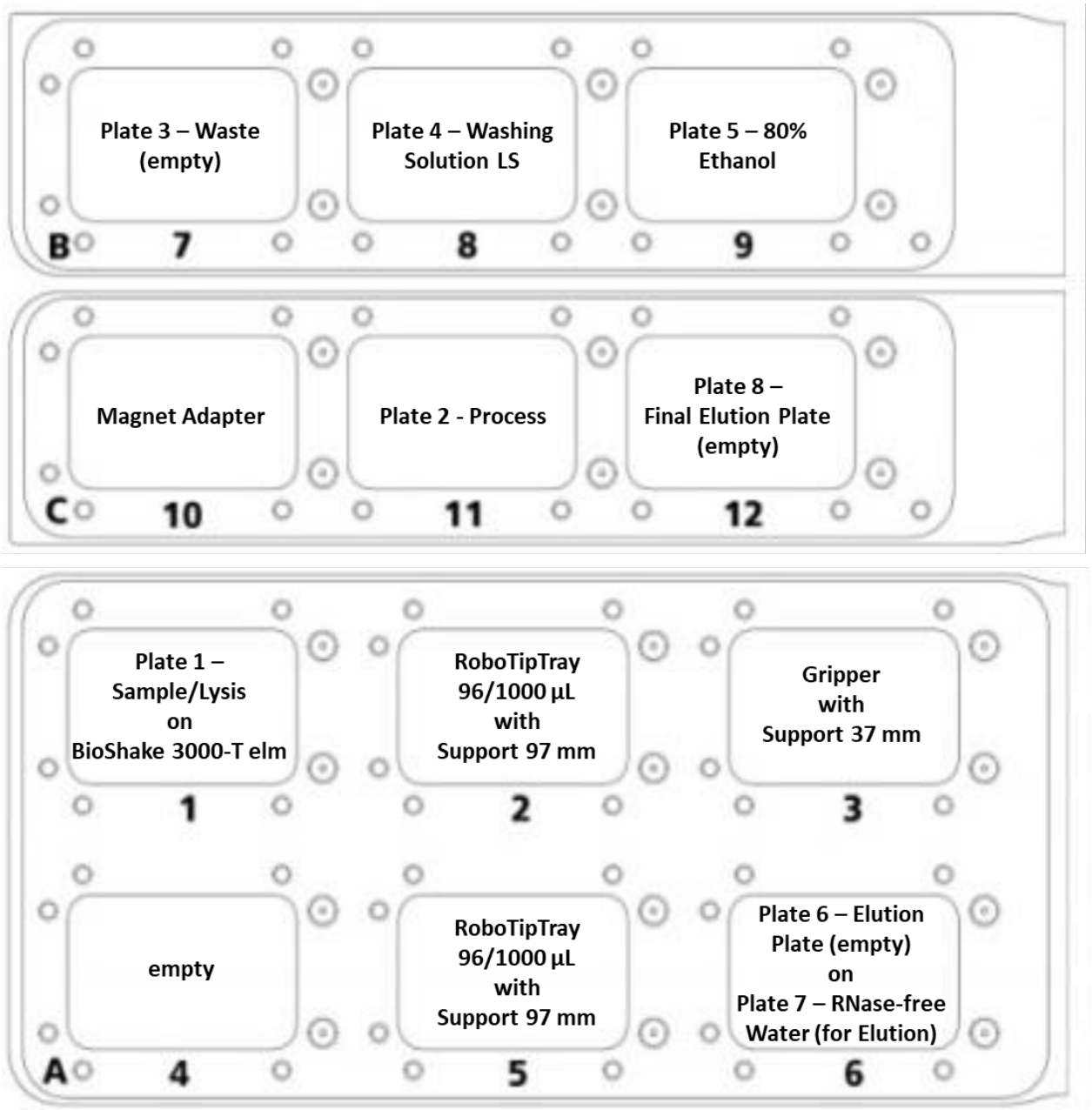

Figure 11: Deck layout for extraction.

2. Switch on the CyBio FeliX and open the "AppStudio FeliX *eXtract*".
3. Select "Magnetic Beads" (→ see Figure 11).

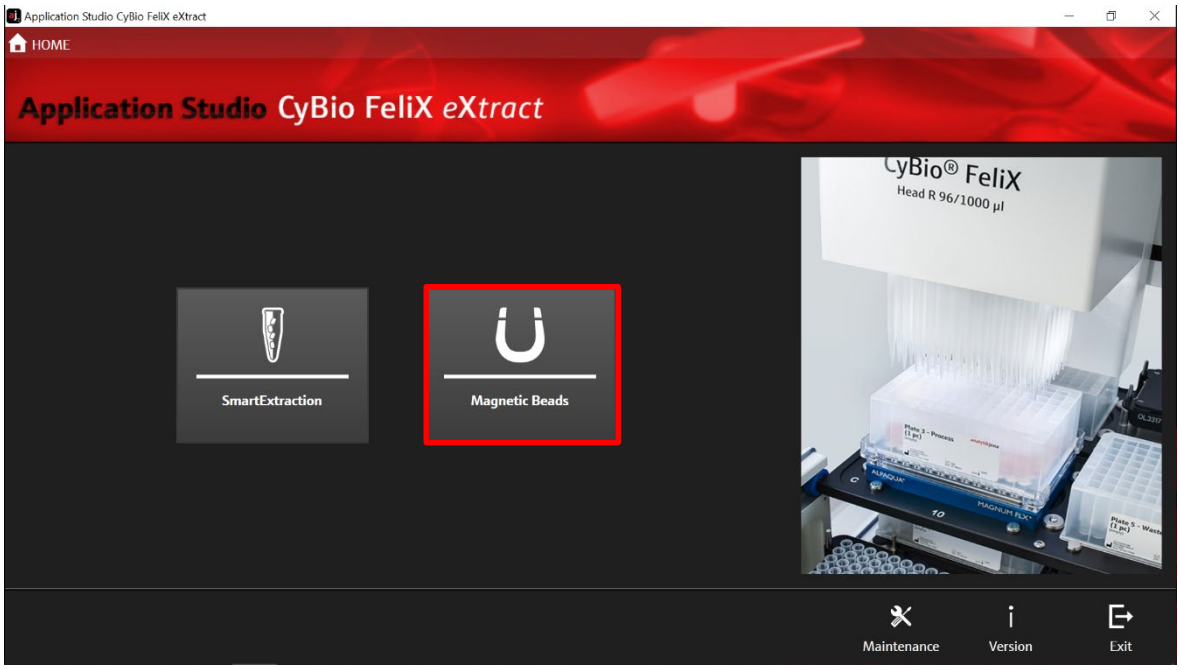

Figure 12: Selection of the Magnetic Beads protocol.

4. Select "innuPREP Virus TS RNA Kit 2.0 – FX" (→ see Figure 12).

Figure 13: Selection of the extraction kit.

5. Select “Extraction” (→ see Figure 13).

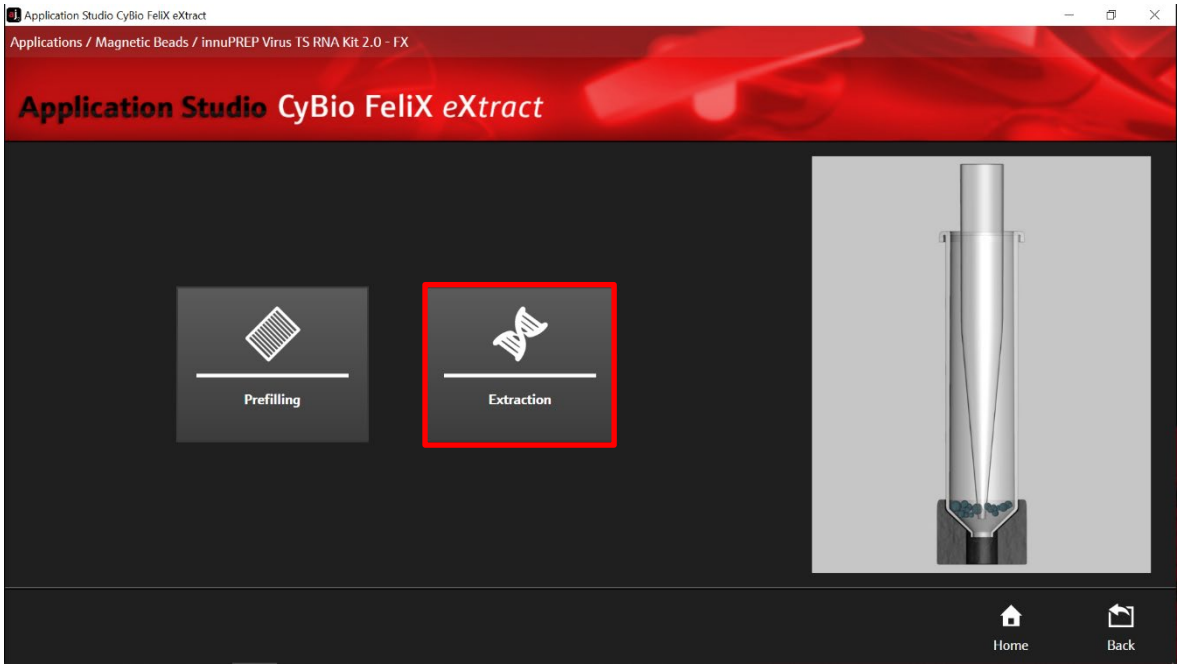

Figure 14: Routine selection in the AppStudio FeliX eXtract: Extraction.

6. Check the correct version number of the protocol (→ see Figure 14). “Extraction - innuPREP Virus TS RNA 2.0 – 02”.

Figure 15: Version number of the extraction protocol.

7. Adjust the elution volume (elution volumes can range from 50 µL to 200 µL, 100 µL are recommended) (→ see Figure 15).

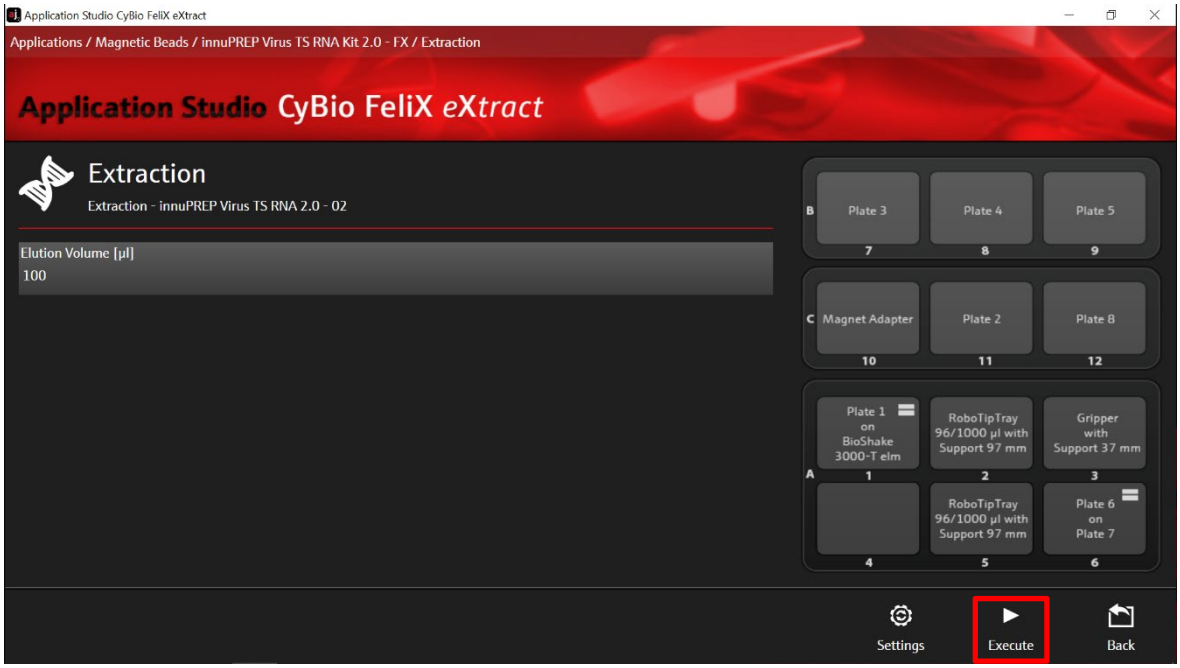

Figure 16: Adjustment of the elution volume.

8. After selecting “Execute” the screen with the deck layout appears. Check the correct positioning of the plates and accessories referring to the deck layout above (→ see Figure 10) and confirm with “Ok” to start the protocol (→ see Figure 16).

Figure 17: Deck layout for checking the correct positions of plates and accessories

9. Confirm the “Process completed” message with “Ok” (→ see Figure 17).

Figure 18: Process completed.

10. Remove **Plate 8 – Final Elution Plate** from **deck position 12** and seal it with the included sealing film. Store the RNA under adequate conditions.

NOTE

When using alternate elution vessels as listed in section 11.2 “Loading of CyBio FeliX” (→ see p. 24), proceed analogously.
Store RNA under adequate conditions. We recommend storing the extracted nucleic acids at -22 °C to -18 °C. For long-term storage we recommend -80 °C.

11. After finishing the extraction protocol, remove and discard the used Deep Well Plates and the used tips.

12 Protocol 2: Isolation directly from the swab samples

NOTE
To avoid mix-ups of samples, prepare a sample layout to assign the individual specimen to a well of the 96-well plate.

12.1 Preparing Process Plate & Sample / Lysis-Plate

NOTE
For the extraction of nucleic acids from swab samples we recommend the addition of Carrier RNA. Ensure the **Carrier RNA** has been prepared as described (→ see section 9 "Initial steps before starting", p. 13). Lysis of the sample material is done automatically and is included in the CyBio FeliX extraction protocol.

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

NOTE
Step 5 does not have to be done when the plates were prefilled with the CyBio FeliX.

1. Place the swabs into 1.5 mL reaction tubes containing **600 µL Lysis Solution CBV** and incubate them for 10 min under continuous shaking.
2. Squeeze the swab and remove it from the reaction tube.
3. Transfer **400 µL** of the **liquid samples** into the desired cavities of **"Plate 1 – Sample / Lysis"** and **add 10 µL Carrier RNA**. Please adhere to your sample layout.
4. Add **10 µL Proteinase K** into the desired cavities of **"Plate 1 – Sample / Lysis"**.
5. Transfer **50 µL of MAG Suspension** directly into the liquid of the prefilled plate **"Plate 2 – Process"**.

NOTE
The sample will be processed using the CyBio FeliX. Please follow the instructions of section 12.2 “Loading of CyBio FeliX” below.

12.2 Loading of the CyBio FeliX

1. Load all plates and accessories according to the scheme below (→ see Figure 18).
   As a Final Elution Plate (**Position 12**) multiple options are possible:
   - Plate 8 - Final Elution Plate
   - Micronic 750 µL pre-capped and racked 2D-tubes (MP52706-Y20)
   - Greiner Cryo.S 600 µL pre-racked (977561, 977580)

NOTE
Please pay special attention to the following deck positions:

Position 1: Place **Plate 1 – Sample / Lysis** on the BioShake 3000-T-elm (**deck position 1**).

Position 6: Stack **Plate 6 – Elution (empty)** directly on **Plate 7 – RNase-free Water (for Elution)**.

Position 2 and 5: Put the **Protective Plate** directly on the bottom plate of the **97 mm support**. Fill 96 Filter Tips (or the number of tips required) into the **CyBio RoboTipTray 1-96/1000 µL** using the **Tip Transfer Tool 96/1000 µL** and put it on the **97 mm support**. Make sure that every Filter Tip fits into a cavity of the Protective Plate.

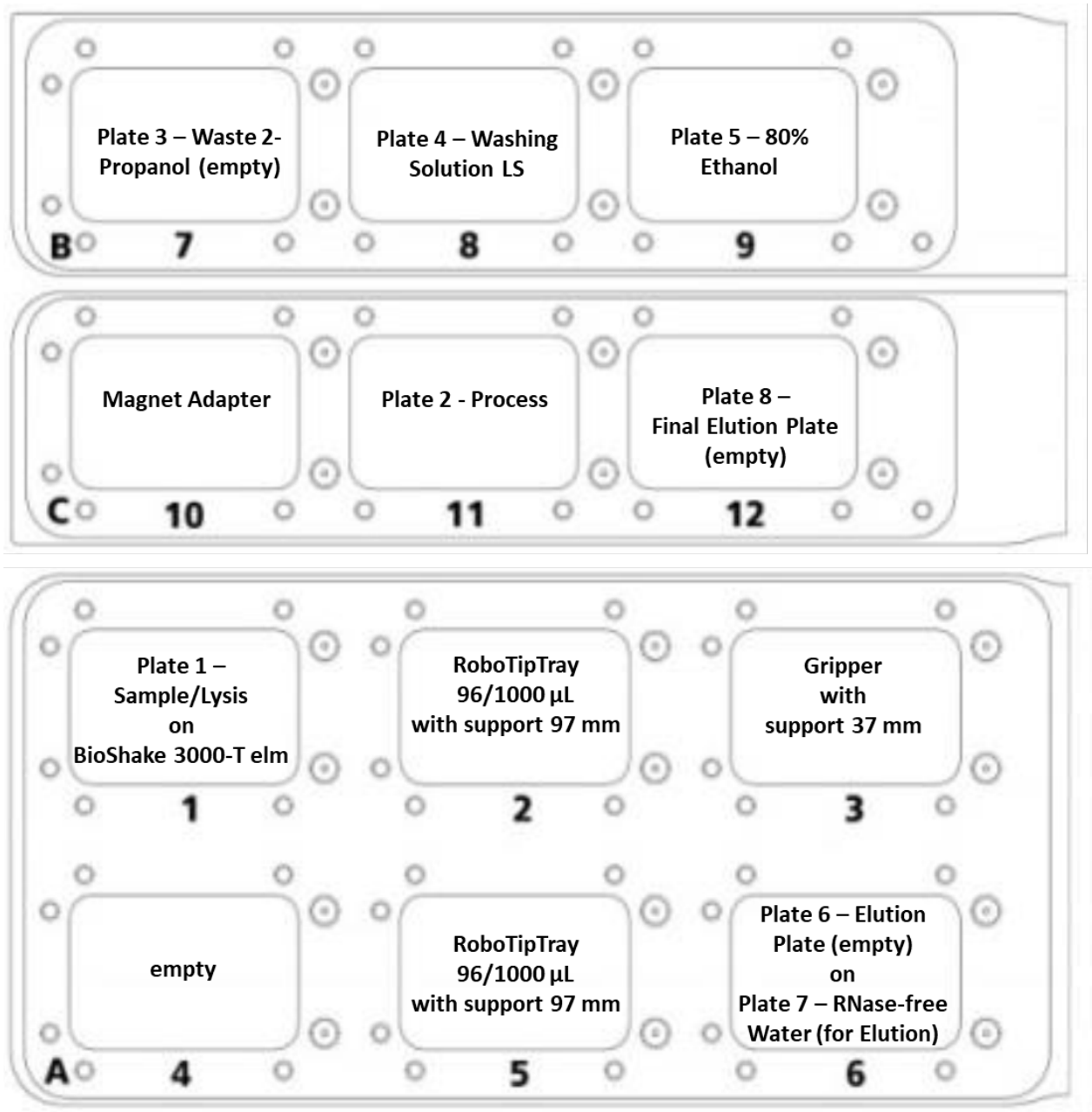

Figure 19: Deck layout for extraction.

2. Switch on the CyBio FeliX and open the "AppStudio FeliX *eXtract*".
3. Select "Magnetic Beads" (→ see Figure 19).

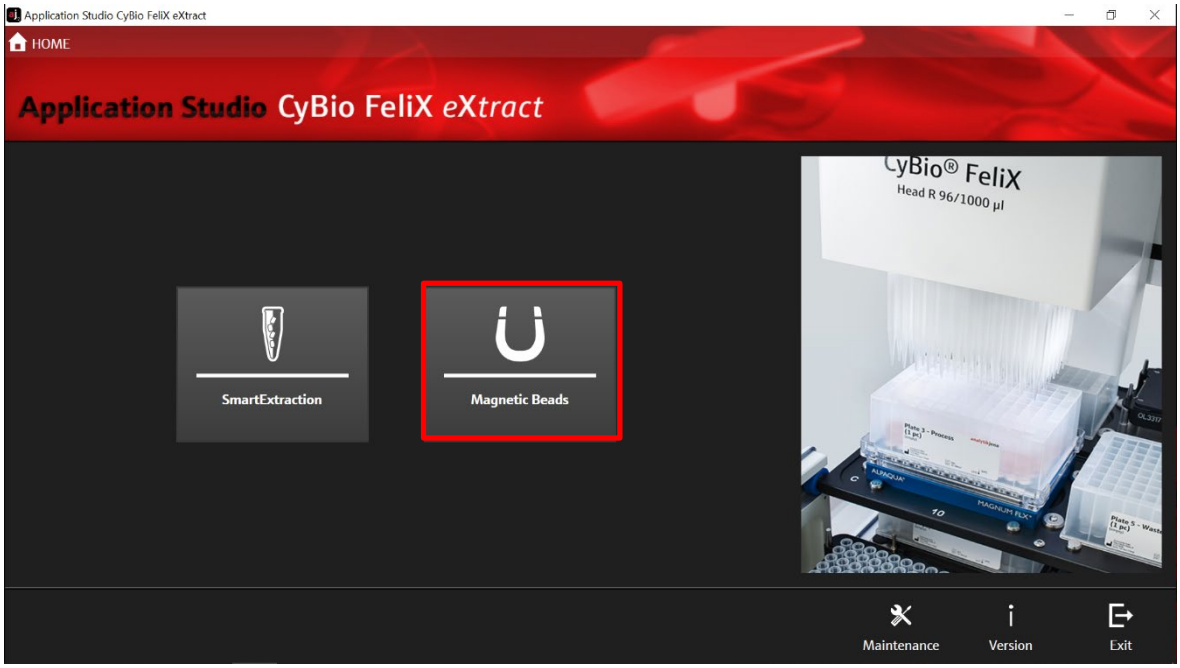

Figure 20: Selection of the Magnetic Beads protocol.

4. Select “innuPREP Virus TS RNA Kit 2.0 – FX” (→ see Figure 20).

Figure 21: Selection of the extraction kit.

5. Select “Extraction” (→ see Figure 21).

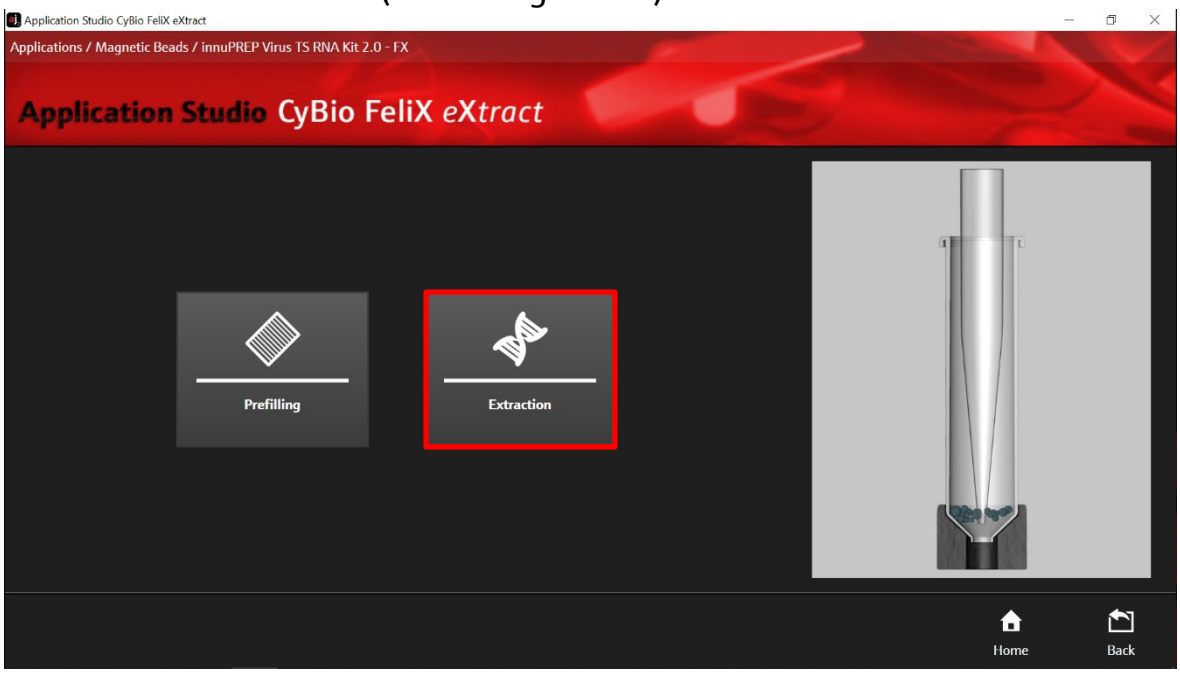

Figure 22: Routine selection in the AppStudio FeliX eXtract: Extraction.

6. Check the correct version number of the protocol (→ see Figure 22). “Extraction - innuPREP Virus TS RNA 2.0 – 02”.

Figure 23: Version number of the extraction protocol and elution volume.

7. Adjust the elution volume (elution volumes can range from 50 µL to 200 µL, 100 µL are recommended) (→ see Figure 23).

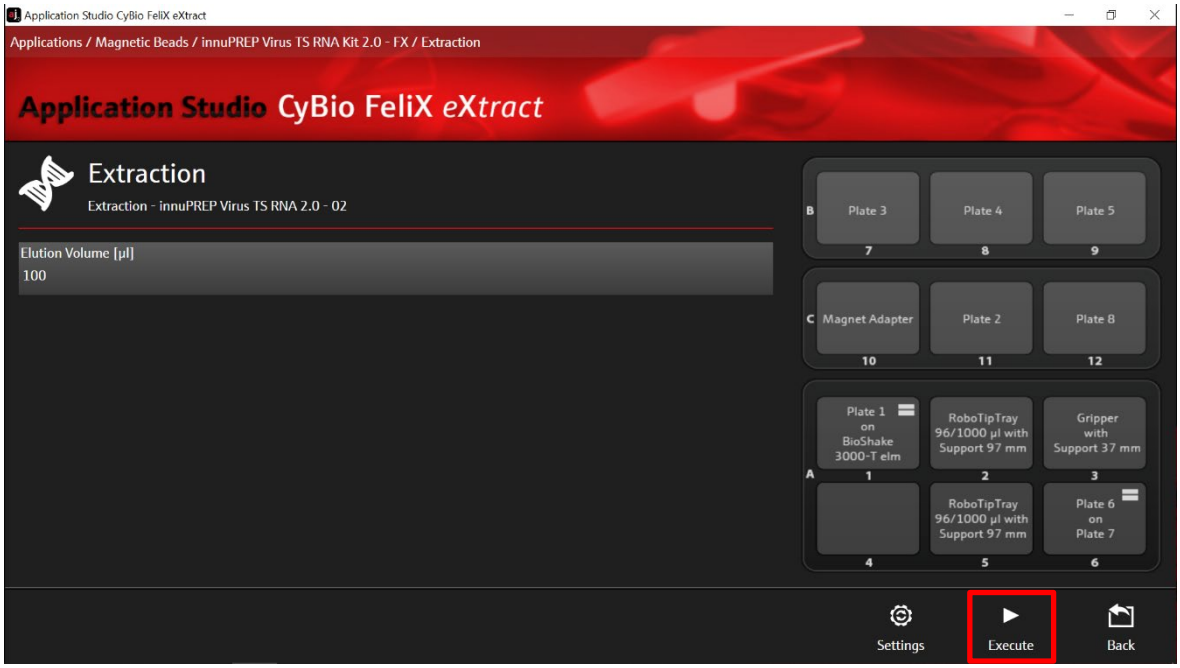

Figure 24: Adjustment of the elution volume.

8. After selecting “Execute” the screen with the deck layout appears. Check the correct positioning of the plates and accessories referring to the deck layout above (→ see Figure 18) and confirm with “Ok” to start the protocol (→ see Figure 24).

Figure 25: Deck layout for checking the correct positions of plates and accessories.

9. Confirm the “Process completed” message with “Ok” (→ see Figure 25).

Figure 26: Process completed.

10. Remove **Plate 8 – Final Elution Plate** from **deck position 12** and seal it with the included sealing film. Store the RNA under adequate conditions.

NOTE

When using alternate elution vessels as listed in section 12.2 “Loading of CyBio FeliX” (→ see p. 31), proceed analogously.

Store DNA/RNA under adequate conditions. We recommend storing the extracted nucleic acids at -22 °C to -18 °C. For long-term storage we recommend -80 °C.

11. After finishing the extraction protocol, remove and discard the used Deep Well Plates and the used tips.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted viral RNA	
Insufficient lysis of starting material.	Ensure that the required volume of 10 µL Proteinase K is used.
Eluate volume too high.	Decrease the elution volume. The recommended elution volume is 100 µL. Please note that reduced elution volume will not necessarily increase the concentration proportionally!
Inadequate extraction. / Inhibitory substances in starting material.	Please use the kit only for samples that match the requirements declared in “Product specifications” (→ see section 8, p. 14). Use Internal Controls for verification of the extraction procedure.

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