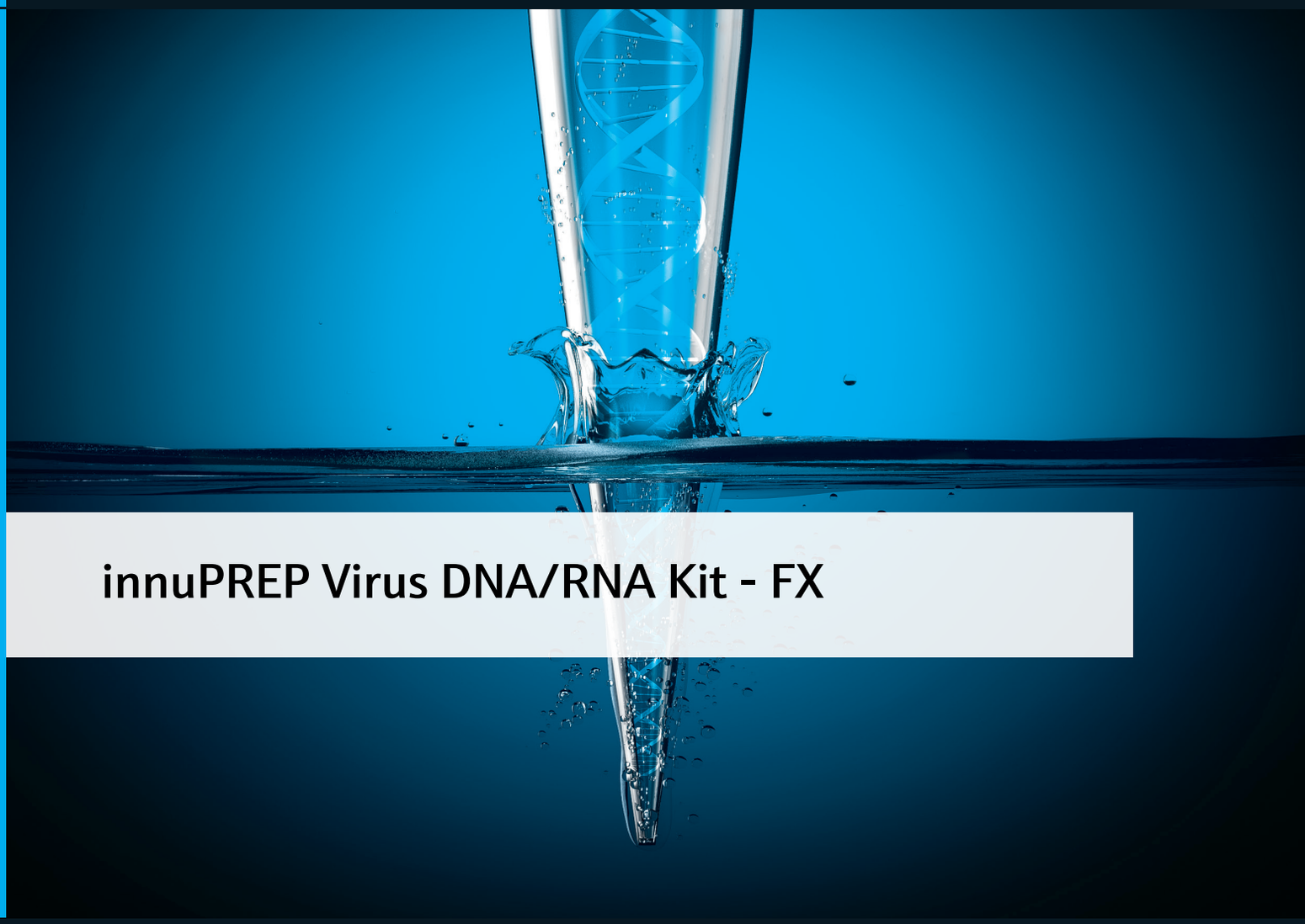
**Instructions for Use**
Life Science Kits & Assays



# innuPREP Virus DNA/RNA Kit - FX

Innuscreen GmbH

**Order No.:**
845-FX-2096096 96 reactions
845-FX-2096480 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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# Contents

# 1 Introduction

## 1.1 Intended use

The **innuPREP Virus DNA/RNA Kit – FX** has been designed for the completely automated isolation of both viral DNA and RNA from 200 µL and 400 µL sample volumes. 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 the DNA and/or 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 Mix with Carrier RNA, Internal Control RNA (IC RNA) and Internal Control DNA (IC DNA) for controlling the extraction process and for better recovery of minute amounts of sample DNA and RNA. Both internal controls can be detected by real-time PCR using the corresponding assays. The addition of individual internal controls needs to be tested carefully. No data are available on the rate of recovery of individual internal controls. Therefore, no guarantee for their recovery can be given. Interference of the kit's Carrier Mix and individual internal control needs to be excluded.

Please note that the eluates of the kit contain both, sample nucleic acids and Carrier Mix. Therefore, it is not possible to quantify the isolated nucleic acids by photometric or fluorometric methods when using the Carrier Mix. 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 the 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**<br>Catalogue number. |
| Σ N | **Content**<br>Contains sufficient reagents for <N> tests. |
| | **Storage conditions**<br>Store at room temperature or shown conditions respectively. |
| | **Consult instructions for use**<br>This information must be observed to avoid improper use of the kit and the kit components. |
| | **Expiry date** |
| LOT | **Lot number**<br>The number of the kit charge. |
| | **Manufactured by**<br>Contact information of manufacturer. |
| | **For single use only**<br>Do not use components for a second time. |
| | **Note / Attention**<br>Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results. |

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → “Notes on the use of this manual”, p. 4).
- Working steps are numbered.

# 2 Safety precautions

**NOTE**
Read through this chapter carefully 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 usage of 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 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 V** and **Carrier Mix** (→ "Preparation of Lysis Solution V / Carrier Mix", p. 25 and p. 42) 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 – 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 DNA/RNA Kit – FX** or other IST Innuscreen 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 use with other starting materials or other amounts of starting material than those, referred to in the manual (→ see “Product specifications”, p. 12). 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 480 |
|---|---|---|
| REF | 845-FX-2096096 | 845-FX-2096480 |
| **MAG Suspension** | 9 mL | 5 x 9 mL |
| **Proteinase K** | for 4 x 1.5 mL working solution | for 17 x 1.5 mL working solution |
| **Carrier Mix** | for 2 x 1.25 mL working solution | for 8 x 1.25 mL working solution |
| **Lysis Solution V** | 110 mL | 5 x 110 mL |
| **Binding Solution V** | 100 mL | 5 x 100 mL |
| **RNase-free Water** | 10 mL | 2 x 25 mL |
| **RNase-free Water (for Elution)** | 70 mL | 5 x 70 mL |
| **Washing Solution A** | 120 mL | 5 x 120 mL |
| **Washing Solution B2 (conc.)** | 48 mL | 5 x 48 mL |
| **Deep Well Plate (square, 2.0 mL)** | 7 | 35 |
| **Final Elution Plate** | 1 | 5 |
| **Protective Plate** | 2 | 10 |
| **Sealing Foil** | 1 | 5 |
| **Filter Tips** | 2 x 96 | 10 x 96 |
| **Manual** | 1 | 1 |

## 7.2 Components not included

- 96–99.8 % Ethanol (molecular biology grade, undenatured) for dilution of Washing Solution B2 (conc.)
- 1.5 mL and 15 mL tubes for swab incubation
- Physiological saline for swab incubation
- Pipetting tips for reagent prefilling
- 2 column and 12 column reservoirs for prefilling by CyBio FeliX (innuPREP Prefilling Set, OL3317-25-127, Analytik Jena GmbH)

## 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)
- Final Elution Plate (96 well, 1.2 mL) (31-01642, 5 pcs, 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:
   - 200 µL or 400 µL cell-free body fluids (serum, plasma)
   - Swabs dry, delivered in physiological saline, liquid VTM, liquid amies or liquid UTM
2. Processing time:

| Sample volume | Automated prefilling | Extraction | Elution volume |
|---|---|---|---|
| 200 µL | 52 min | 71 min | 50–200 µL |
| 400 µL | 60 min | 82 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 1.5 mL dd$H_2O$ to each vial lyophilized **Proteinase K**, mix thoroughly and store as described above.
- Add 1.25 mL RNase-free Water to each vial lyophilized **Carrier Mix,** mix thoroughly and store as described above.
- Add 72 mL absolute ethanol to each bottle **Washing Solution B2 (conc.)** and mix thoroughly. Keep the bottle always firmly closed!
- Prepare **Lysis Solution V / Carrier Mix** as indicated in each protocol.
- Put accessories on the 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) |

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

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 CyBio TipRack 96/1000 µL (right).

# 10 Usage of Carrier Mix

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 |

If customized extraction controls are used, please add these components to the mixture of **Lysis Solution V / Carrier Mix** (→ see sections 12.3 and 14.3 “Preparation of Lysis Solution V / Carrier Mix”, p. 25 and p. 42).

# 11 Prefilling of Reagent Plates for 200 µL sample volume

Plates may be prefilled automatically with the CyBio FeliX (→ see section 11.1) or manually (→ see section 11.2).

## 11.1 Automated prefilling with CyBio FeliX

**NOTE**
For 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).

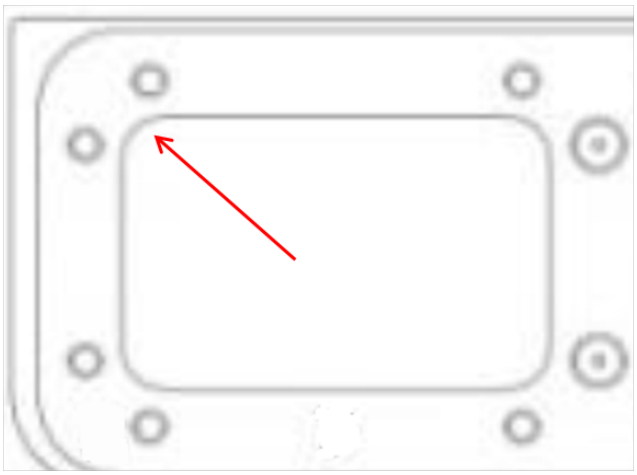

**Figure 2: Positioning of plates and reservoirs on CyBio FeliX deck.**

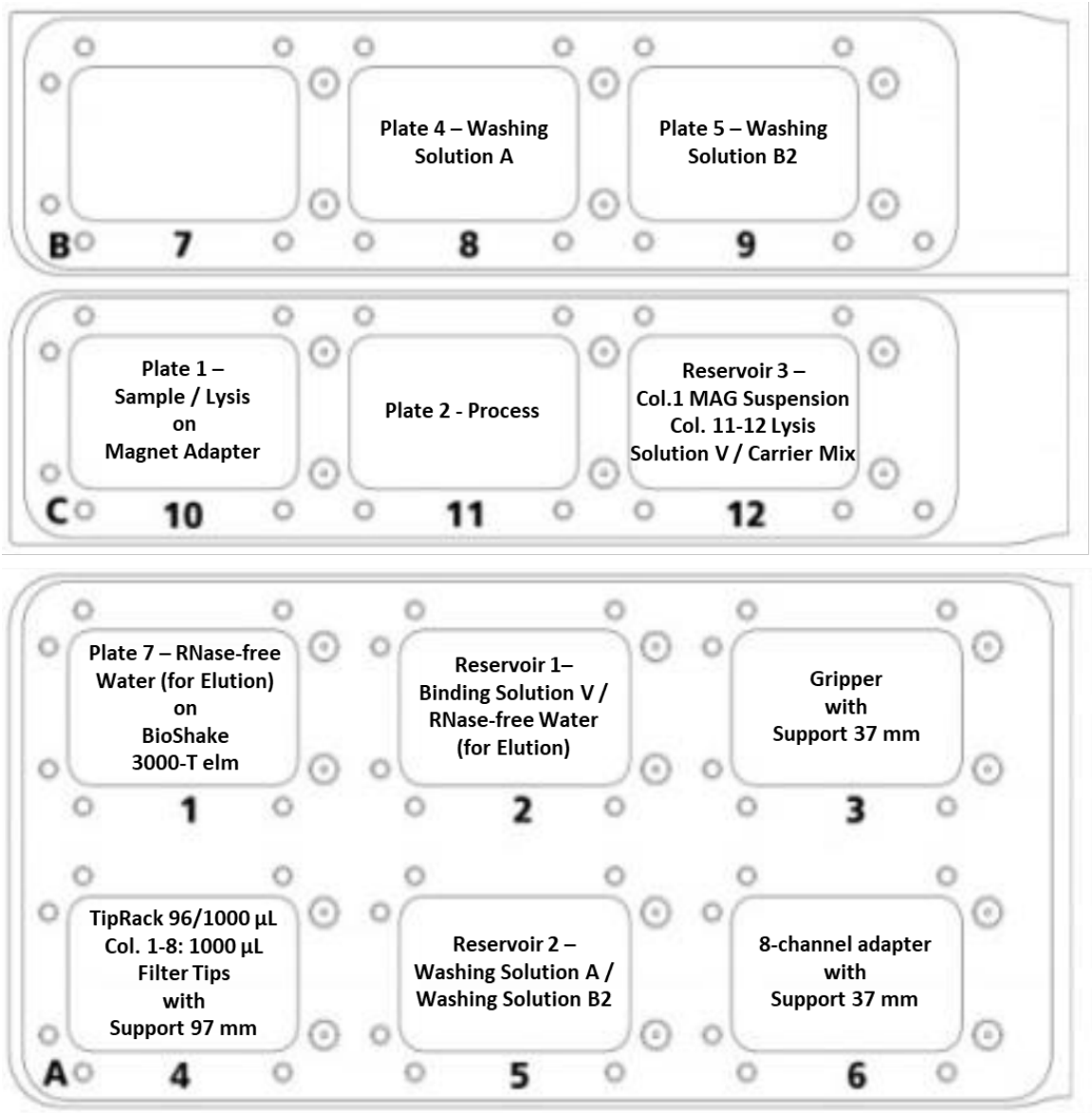


Figure 3: Deck layout for starting the prefilling protocol for 200 µL sample volume.

**NOTE**

Due to interactions of **MAG Suspension** with buffer components, 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.

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<br>(2 column) | **Reservoir 1:**<br>Left side of reservoir: Binding Solution V<br>Right side of reservoir: RNase-free Water (for Elution) |
| Reservoir 2<br>(2 column) | **Reservoir 2:**<br>Left side of reservoir: Washing Solution A<br>Right side of reservoir: Washing Solution B2 |
| Reservoir 3<br>(12 column) | **Reservoir 3:**<br>Column 1 MAG Suspension<br>Column 11-12 Lysis Solution V / Carrier Mix |

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 A |
| **Plate 5** | Washing Solution B2 |
| **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 the content of one bottle (100 mL) **“Binding Solution V”** into the **left** side of the 2 column reservoir labeled “Reservoir 1 - Binding Solution V / RNase-free Water (for Elution)”.

4. Transfer the content of one bottle (70 mL) **"RNase-free Water (for Elution)"** into the **right** side of the 2 column reservoir labeled "Reservoir 1 - Binding Solution V / RNase-free Water (for Elution)". Place the filled reservoir into the CyBio FeliX on position 2 (→ see Figure 3).
5. Transfer the content of one bottle (120 mL) **"Washing Solution A"** into the **left** side of the 2 column reservoir labeled "Reservoir 2 – Washing Solution A / Washing Solution B2".
6. Transfer the content of one bottle (120 mL) **"Washing Solution B2"** into the **right** side of the 2 column reservoir labeled "Reservoir 2 – Washing Solution A / Washing Solution B2. Place the filled reservoir into the CyBio FeliX on position 5 (→ see Figure 3).
7. Vortex the **MAG Suspension** properly (at least 30 s). Transfer the complete content of the bottle (9 mL) into column 1 of the reservoir labeled "Reservoir 3 – MAG Suspension / Lysis Solution V / Carrier Mix".
8. Transfer 15 mL of the prepared **Lysis Solution V / Carrier Mix** (→ "Preparation of Lysis Solution V / Carrier Mix", p. 25 and p. 42) in column 11 and 15 mL in column 12 of the reservoir labeled "Reservoir 3 – MAG Suspension / Lysis Solution V / Carrier Mix". Place the filled reservoir into the CyBio FeliX on position 12 (→ see Figure 3).
9. Insert Filter Tips in columns 1 - 8 in the CyBio TipRack 96/1000 µL. Please fill these columns completely with Filter Tips.
10. Place the CyBio Tip Rack 96/1000 µL into the CyBio FeliX on position 4 (→ see Figure 3).
11. Place the 8-channel adapter (Head R 96) with the support 37 mm into the CyBio FeliX on position 6 (→ see Figure 3).
12. Place the empty, labeled plates on the CyBio FeliX deck according to the deck layout for the prefilling protocol for 200 µL sample volume (→ see Figure 3).

**NOTE**
Please pay special attention to the following deck positions:

Position 1:
Place **Plate 7 – RNase-free Water (for Elution)** directly on the BioShake 3000-T elm.

Position 10:
Place **Plate 1 – Sample / Lysis** on the Magnet Adapter.

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

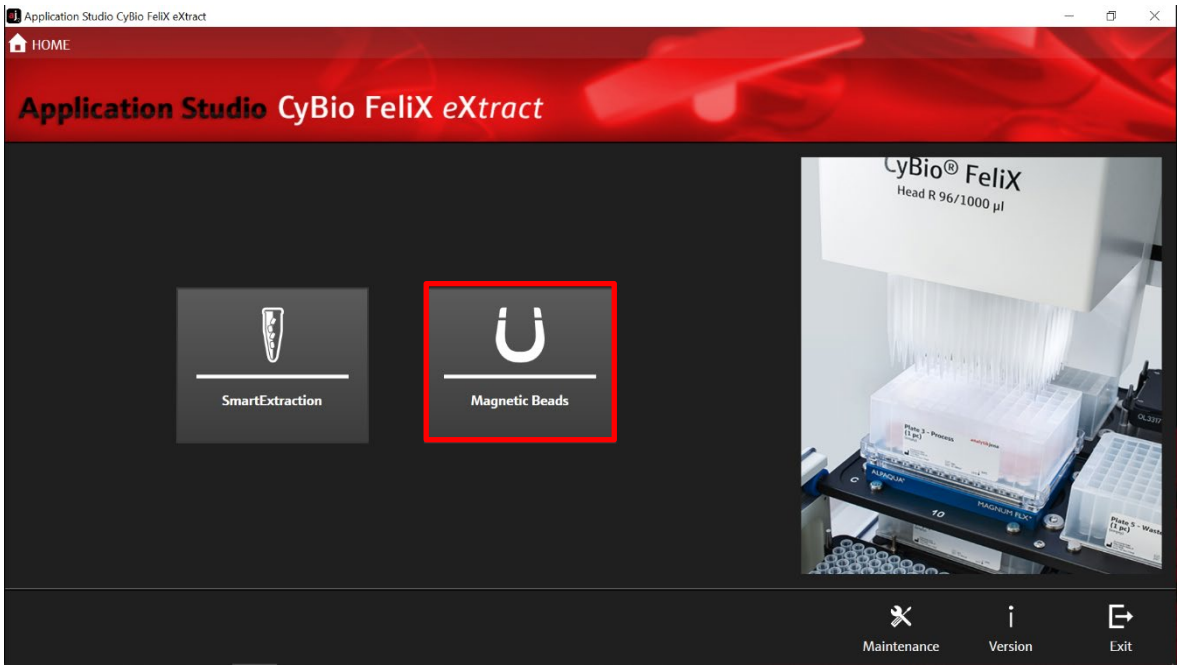


**Figure 4: Homescreen of the AppStudio FeliX eXtract.**

15. Choose "innuPREP Virus DNA/RNA Kit – FX" (→ see Figure 5).


**Figure 5: Kit selection.**

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

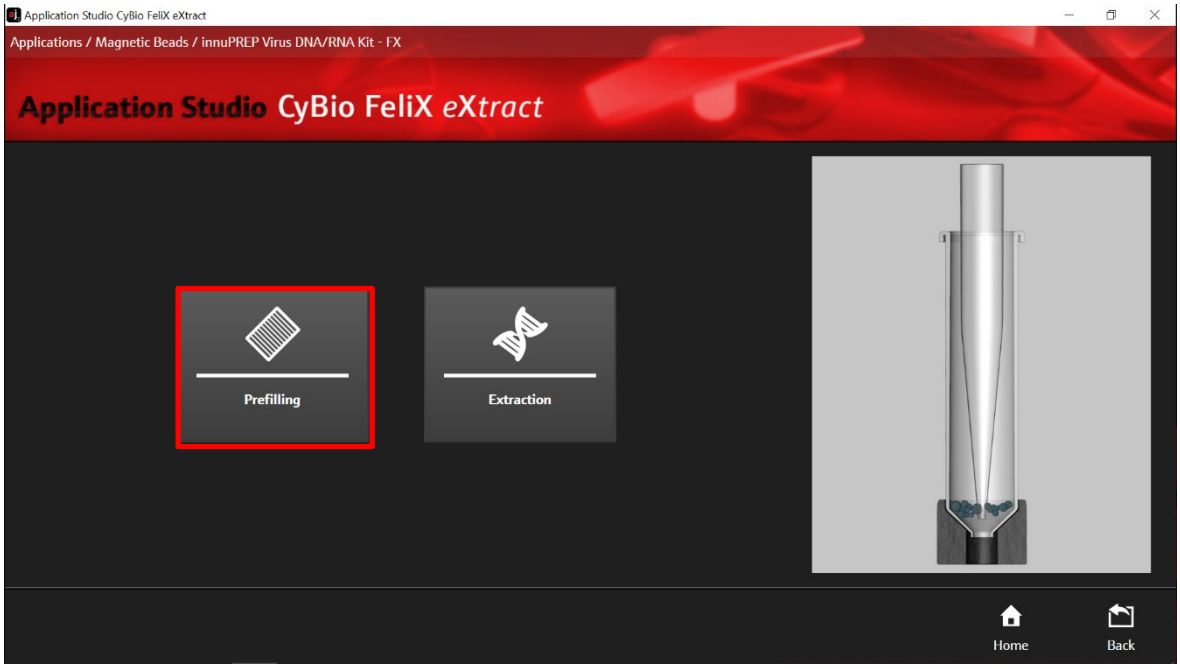


**Figure 6: Routine selection: Prefilling.**

17. After choosing “Prefilling” the Prefilling Start Screen appears.

18. Check the correct version number of the protocol (→ see Figure 7): “Prefilling – innuPREP Virus DNA/RNA – 02”.


**Figure 7: Version number of the prefilling protocol.**

19. To adjust the sample volume, click the button “Sample Volume”. Choose “200 µL” and confirm with “Execute” (→ see Figure 8).

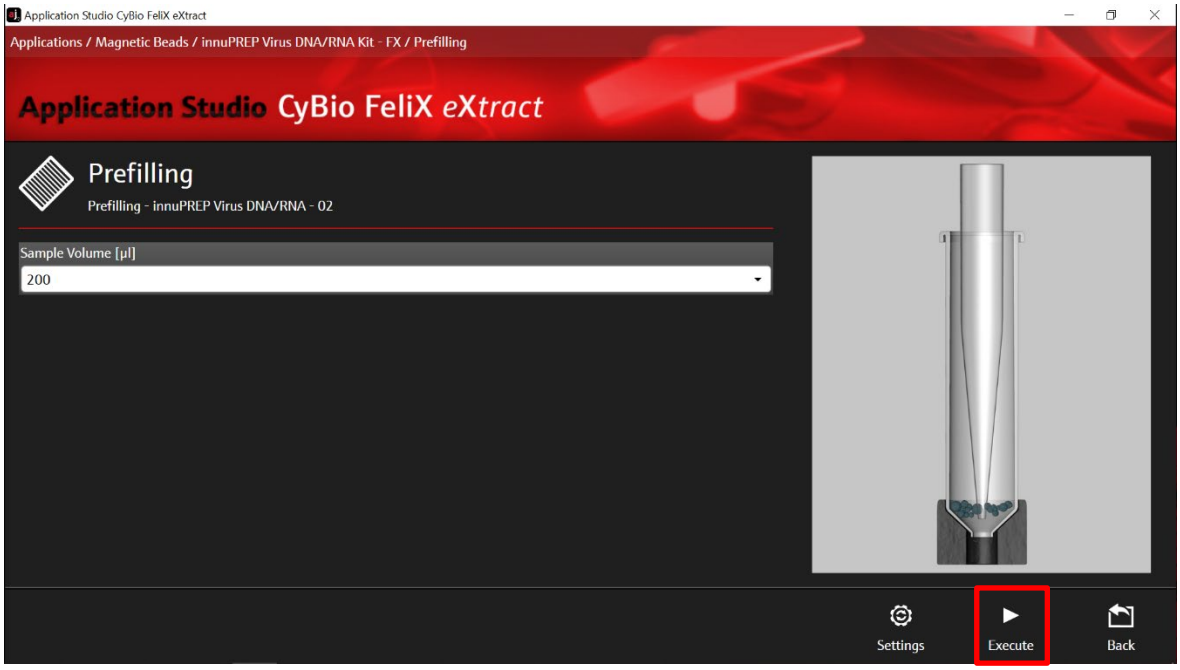


**Figure 8: Selection of the sample volume.**

20. Check the correct deck position of all plates, reservoirs and other hardware components (compare with list displayed in the AppStudio FeliX *eXtract* → see Figure 9) and confirm with “Ok”.


**Figure 9: Deck layout for final hardware check for the prefilling.**

21. 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 10).


**Figure 10: Prefilling completed.**

22. Remove the CyBio TipRack 96/1000 µL and discard all tips.
23. Remove 8-channel adapter (Head R 96) with Support 37 mm.

24. Discard the reservoirs and their contents.
25. Remove **Plate 1 – Sample / Lysis** from position 10 and prepare the plate for extraction (see section 12.3 “Preparing the Process Plate & Sample / Lysis Plate”, p. 26).
26. The plates **Plate 3 – Binding Solution V, Plate 4- Washing Buffer A, Plate 5 – Washing Buffer B2, Plate 2 – Process** and **the Gripper on Support 37 mm** do not have to be removed for the extraction process.
27. **Plate 7 – RNase free Water (for Elution)** has to be removed from position 1 and placed on position 6.

## 11.2 Manual prefilling for 200 µL sample volume

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

| Plate | Label | Content per well |
|---|---|---|
| **Plate 1*** | Sample / Lysis | empty (→ see section 12.2 “Sample preparation (200 µL)”, p. 25) |
| **Plate 2** | Process | **450 µL** Binding Solution V |
| **Plate 3*** | Waste | empty |
| **Plate 4** | Washing Solution A | **1100 µL** Washing Solution A |
| **Plate 5** | Washing Solution B2 | **1100 µL** Washing Solution B2 |
| **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.

# 12 Extraction from 200 µL sample volume

**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 Lysis Solution V / Carrier Mix

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

| Component | 96 samples |
|---|---|
| **Lysis Solution V** | 35 mL |
| **Carrier Mix** | 1.75 mL |
| **Final volume** | 36.75 mL |

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

## 12.2 Sample preparation (200 µL)

**NOTE**
For the extraction of nucleic acids from swab samples we recommend the addition of Carrier Mix. Make sure that the **Carrier Mix** has been prepared as described (→ see section 9 "Initial steps before starting", p. 12). Ensure also that the **Lysis Solution / Carrier Mix** has been prepared according the instructions given above. Lysis of the sample material is done automatically and is included in the CyBio FeliX extraction protocol.

1. **Swabs stored in a storage solution/transport medium**
   Shake the swab vigorously within the solution in the storage tube, 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** (→ see section 12.3 “Preparing the Process Plate & Sample / Lysis Plate”, p. 26).

2. **Dry swabs**
   Place the swabs into 1.5 mL reaction tubes containing 500 µL physiological saline (0.9 % NaCl, not included in the kit) and incubate while continuously shaking for 20 min.
   Squeeze all liquid from the swab and remove the it from the reaction tube.
   Proceed with **200 µL of the particle-free sample** (→ see section 12.3 “Preparing the Process Plate & Sample / Lysis Plate”, p. 26).

3. **Plasma/serum samples**
   Proceed with **200 µL of the particle-free sample** (→ see section 12.3 “Preparing the Process Plate & Sample / Lysis Plate”, p. 26).

## 12.3 Preparing the Process Plate & Sample / Lysis Plate

**NOTE**
Steps 1 and 2 do 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 V / Carrier Mix** into “**Plate 1 – Sample / Lysis”**.
3. Add **200 µL sample** into the desired cavity of the prefilled plate “**Plate 1 – Sample / Lysis”**. Please adhere to your sample layout.
4. Add **50 µL Proteinase K** into the prefilled cavities of plate “**Plate 1 – Sample / Lysis”**.

**NOTE**
The sample will be processed using the CyBio FeliX. Please follow the instructions of section 12.4 “Loading of CyBio FeliX”, p. 27.

## 12.4 Loading of CyBio FeliX

1. Load all plates and accessories according to the scheme below (→ see Figure 11, p. 28).
   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**
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, p. 15).

**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. Using the Tip Transfer Tool fill 96 Filter Tips (or the number of tips required) into the CyBio RoboTipTray 1-96/1000 µL and put it on the 97 mm support. Make sure that every Filter Tip fits into a cavity of the Protective Plate.

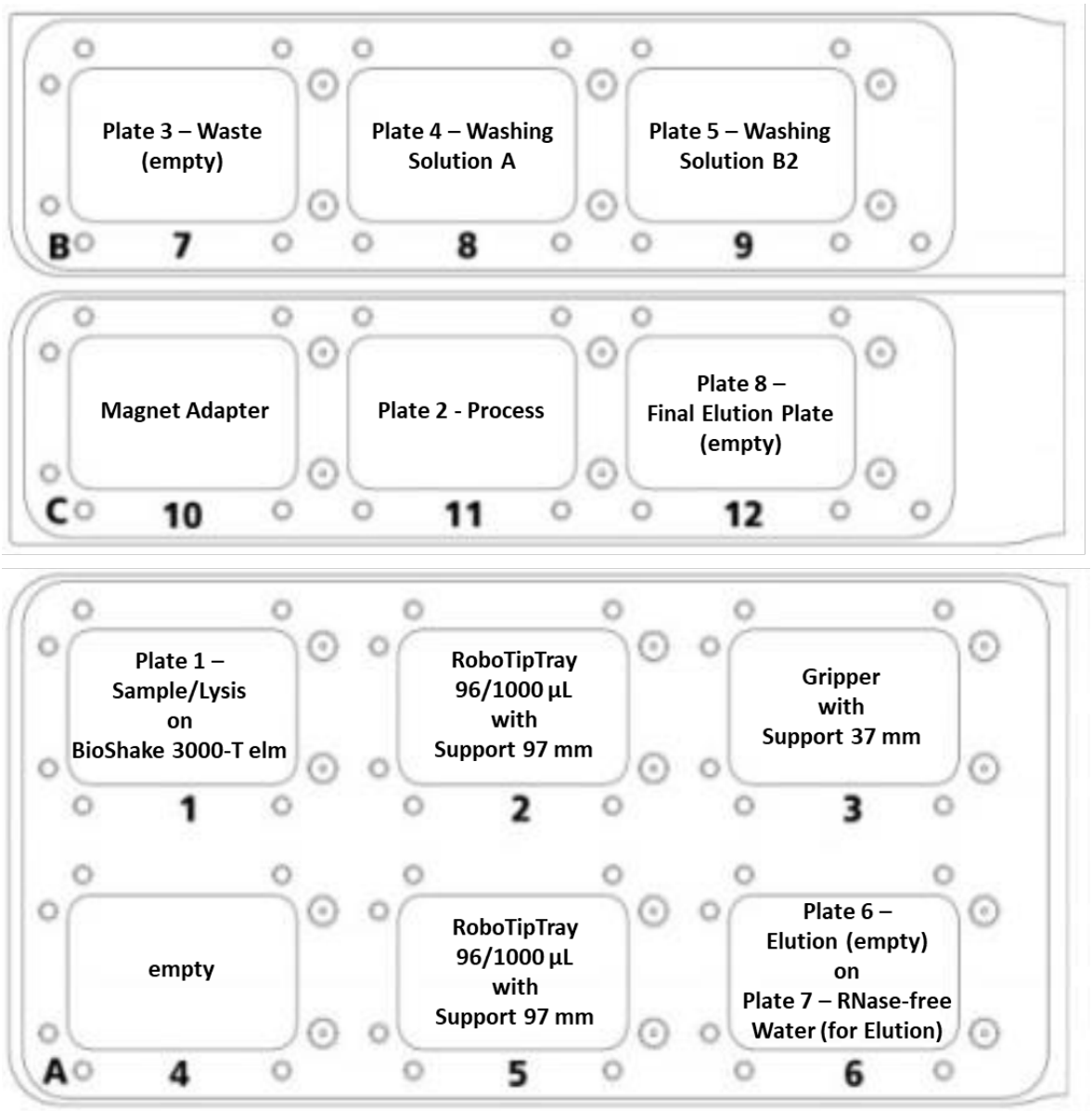


Figure 11: Deck layout for extraction from a 200 µL sample.

2. Switch on the CyBio FeliX and open the "AppStudio FeliX *eXtract*".
3. Select "Magnetic Beads" (→ see Figure 12).

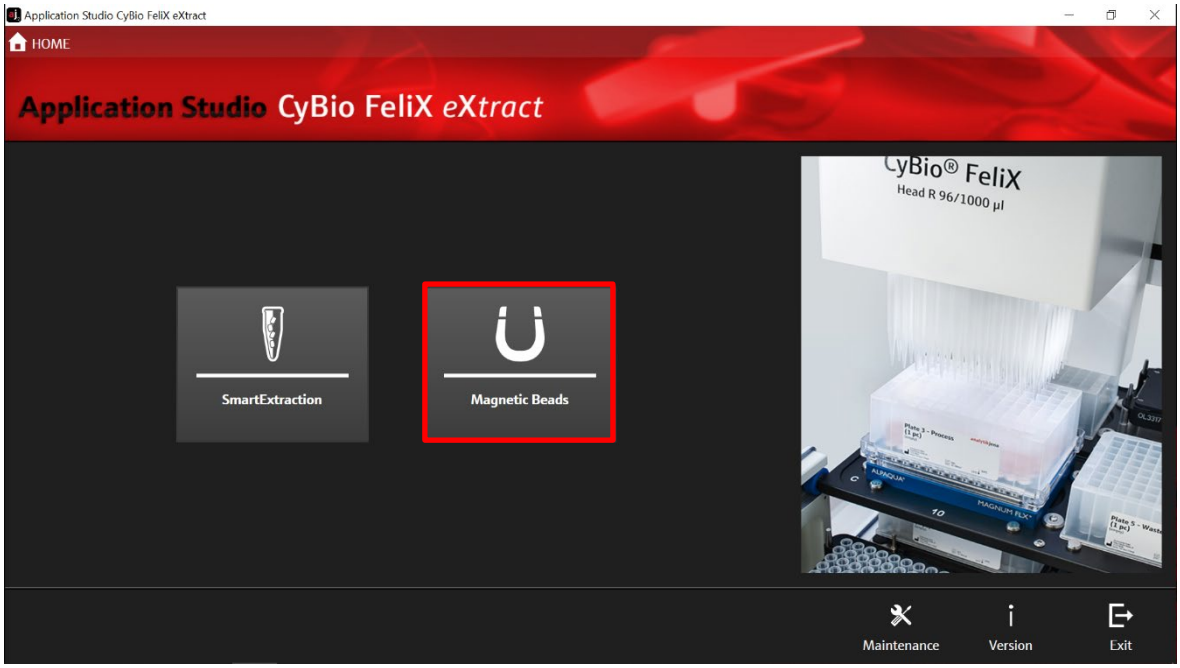


**Figure 12: Selection of the Magnetic Beads Protocol.**

4. Select “innuPREP Virus DNA/RNA Kit – FX” (→ see Figure 13).


**Figure 13: Selection of the extraction kit.**

5. Select “Extraction” (→ see Figure 14).

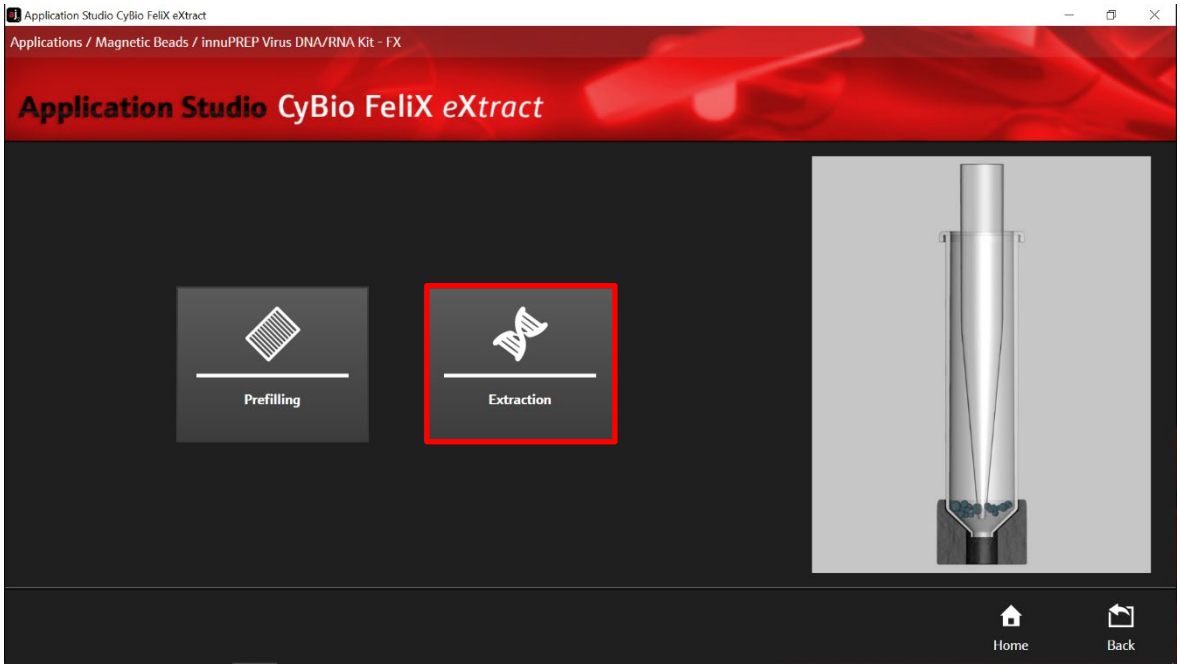


**Figure 14: Routine selection in the AppStudio FeliX eXtract: Extraction.**

6. After selecting “Extraction” the Extraction Start Screen appears (→ see Figure 16).
7. Check the correct version number of the protocol: “Extraction - innuPREP Virus DNA/RNA – 02” (→ see Figure 15).


**Figure 15: Version number of the extraction protocol.**

8. Select the protocol by choosing the corresponding sample volume (200 µL) and adjust the elution volume (between 50–200 µL, 100 µL are recommended) (→ see Figure 16).

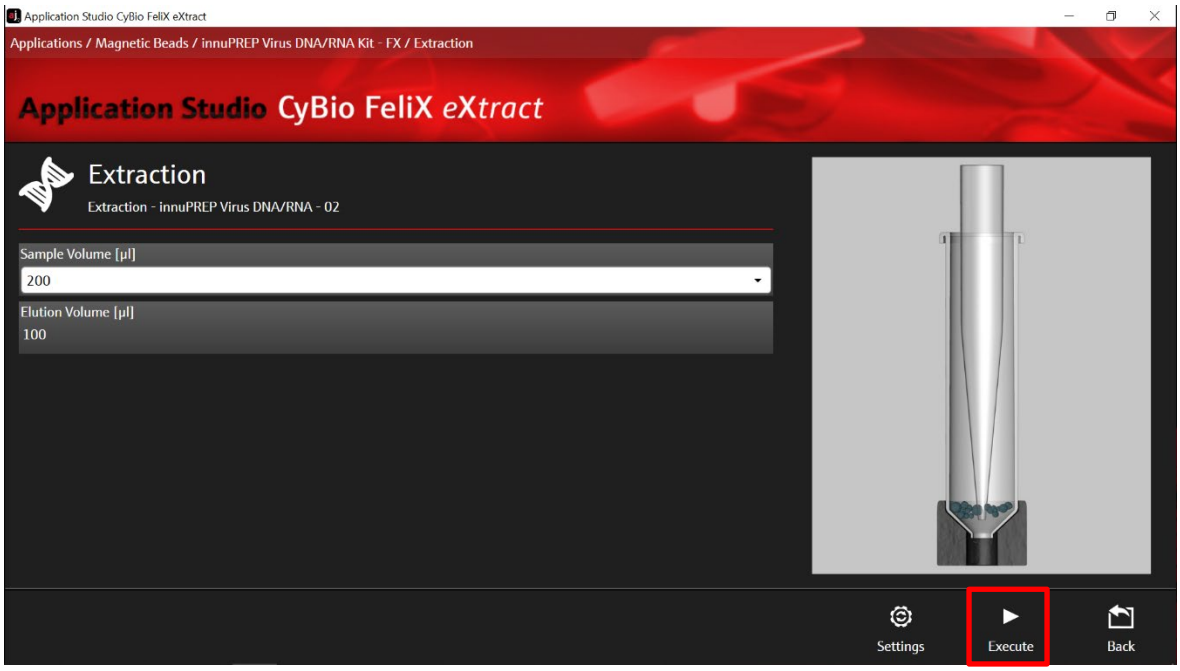


**Figure 16: Selection of sample volume (200 µL) and elution volume (variable).**

9. After selecting “Execute” the deck layout appears. Check the correct positioning of the plates and accessories and confirm with “Ok” to start the protocol (→ see Figure 17).


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

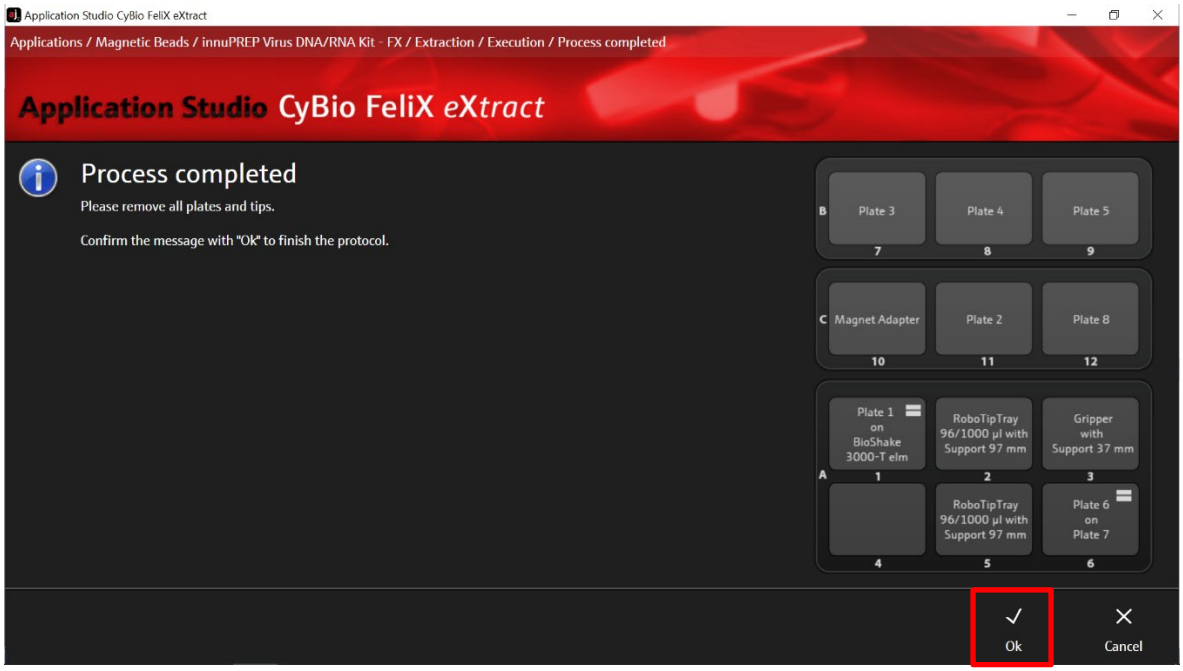


**Figure 18: Process completed.**

10. Confirm the “Process completed” message with “Ok” (→ see Figure 18).
11. Remove **Plate 8 – Final Elution Plate** from **deck position 12** and seal it with the included sealing film. Store the DNA/RNA under adequate conditions.

**NOTE**

When using alternate elution vessels as listed in (→ see section 12.4 “Loading of CyBio FeliX”, p. 27), 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 a storage temperature of -80 °C.

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

# 13 Prefilling of Reagent Plates for 400 µL sample volume

There is the option to prefill the plates automatically with the CyBio FeliX (→ see section 13.1) or manually (→ see section 13.2).

## 13.1 Automated prefilling with CyBio FeliX

**NOTE**
For 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).

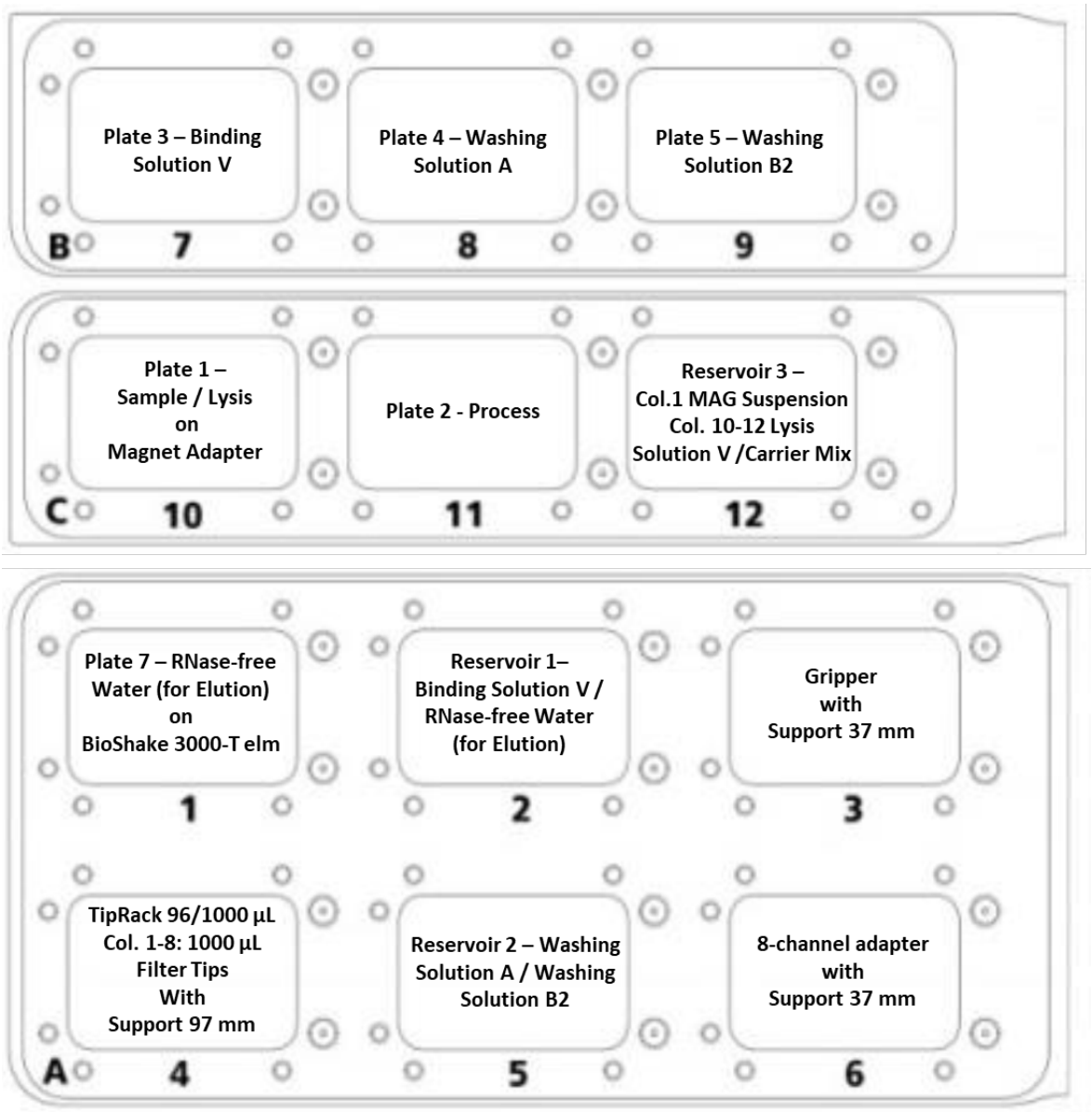


**Figure 19: Deck layout for starting the prefilling protocol for 400 µL sample volume.**

**NOTE**
Due to interactions of MAG Suspension with buffer components, 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.

1. Label three reservoirs (→ see section 7.2 "Components not included", p. 10) according to the table below:

| Number | Label |
|---|---|
| Reservoir 1<br>(2 column) | **Reservoir 1:**<br>Left side of reservoir: Binding Solution V<br>Right side of reservoir: RNase-free Water (for Elution) |
| Reservoir 2<br>(2 column) | **Reservoir 2:**<br>Left side of reservoir: Washing Solution A<br>Right side of reservoir: Washing Solution B2 |
| Reservoir 3<br>(12 column) | **Reservoir 3:**<br>Column 1 MAG Suspension<br>Columns 10-12 Lysis Solution V / Carrier Mix |

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

| Plate | Label |
|---|---|
| **Plate 1** | Sample / Lysis |
| **Plate 2** | Process |
| **Plate 3** | Binding Solution V |
| **Plate 4** | Washing Solution A |
| **Plate 5** | Washing Solution B2 |
| **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 the content of one bottle (100 mL) **"Binding Solution V"** into the **left** side of the 2 column reservoir labeled "Reservoir 1 - Binding Solution V / RNase-free Water (for Elution)".
4. Transfer the content of one bottle (70 mL) **"RNase-free Water (for Elution)"** into the **right** side of the 2 column reservoir labeled "Reservoir 1 - Binding Solution V / RNase-free Water (for Elution)". Place the filled reservoir into the CyBio FeliX on position 2 (→ see Figure 19).
5. Transfer the content of one bottle (120 mL) **"Washing Solution A"** into the **left** side of the 2 column reservoir labeled "Reservoir 2 – Washing Solution A / Washing Solution B2".
6. Transfer the content of one bottle (120 mL) "**Washing Solution B2**" into the right side of the 2 column reservoir labeled "Reservoir 2 – Washing Solution A / Washing Solution B2". Place the filled reservoir into the CyBio FeliX on position 5(→ see Figure 19).
7. Vortex the **MAG Suspension** properly (at least 30 s). Transfer the complete content of the bottle (9 mL) into column A1 of the reservoir labeled "Reservoir 3 – MAG Suspension / Lysis Solution V / Carrier Mix".
8. Transfer 15 mL of the prepared **Lysis Solution V / Carrier Mix** (→ see "Preparing Lysis Solution V / Carrier-Mix, p. 25, p. 42) in column A10, 15 mL in the column A11 and 15 mL in the column A12 of the reservoir labeled "Reservoir 3 – MAG Suspension / Lysis Solution V / Carrier Mix ". Place the filled reservoir into the CyBio FeliX on position 12 (→ see Figure 19).
9. Insert Filter Tips in columns 1-8 in the CyBio TipRack 96/1000 µL. Please fill these columns completely with Filter Tips.
10. Place the CyBio TipRack 96/1000 µL into the CyBio FeliX on position 4 (→ see Figure 19).
11. Place the 8-channel adapter (Head R 96) with the support 37 mm into the CyBio FeliX on position 6 (→ see Figure 19).
12. Place the empty, labeled plates on the CyBio FeliX deck according to the deck layout for the prefilling protocol for 400 µL sample volume (→ see Figure 19).

**NOTE**
Please pay special attention to the following deck positions:

Position 1:
Place **Plate 7 – RNase-free Water (for Elution)** directly on the BioShake 3000-T elm.

Position 10:
Place **Plate 1 – Sample / Lysis** on the Magnet Adapter.

13. Switch on the CyBio FeliX and open AppStudio FeliX *eXtract*.
14. Select "Magnetic Beads" (→ see Figure 20).

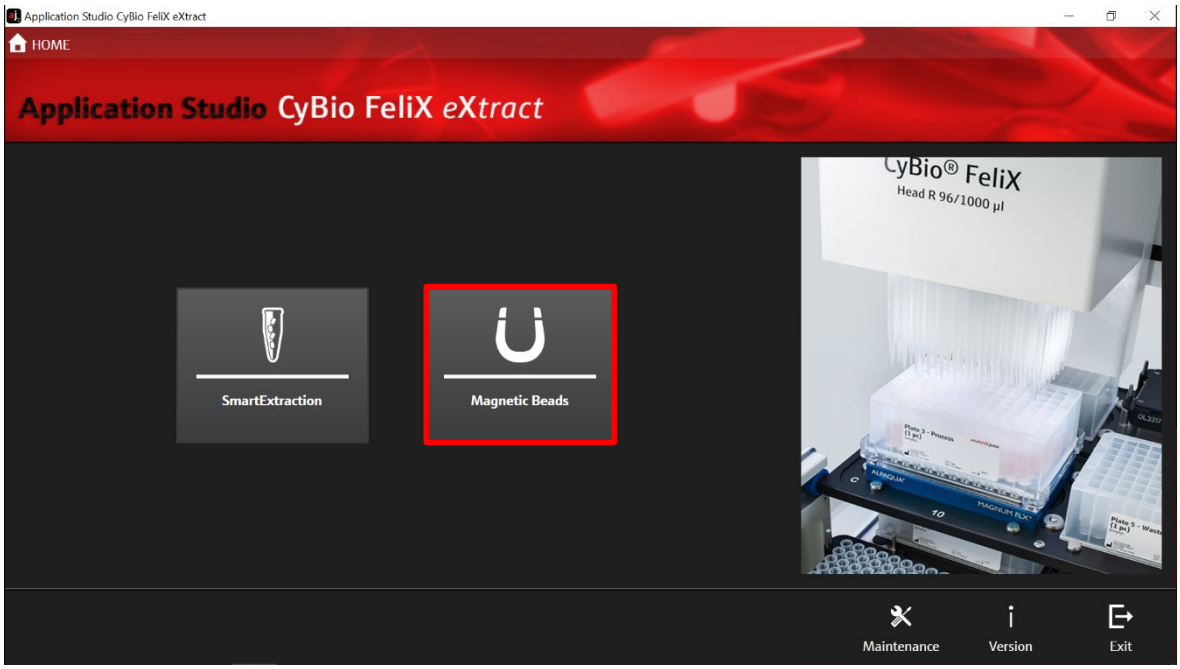


**Figure 20: Home screen of the AppStudio FeliX eXtract. Selection of extraction technology: Magnetic Beads.**

15. Select “innuPREP Virus DNA/RNA Kit –FX” (→ see Figure 21).


Figure 21: Kit selection in the AppStudio FeliX eXtract.

16. Select “Prefilling” (→ see Figure 22).

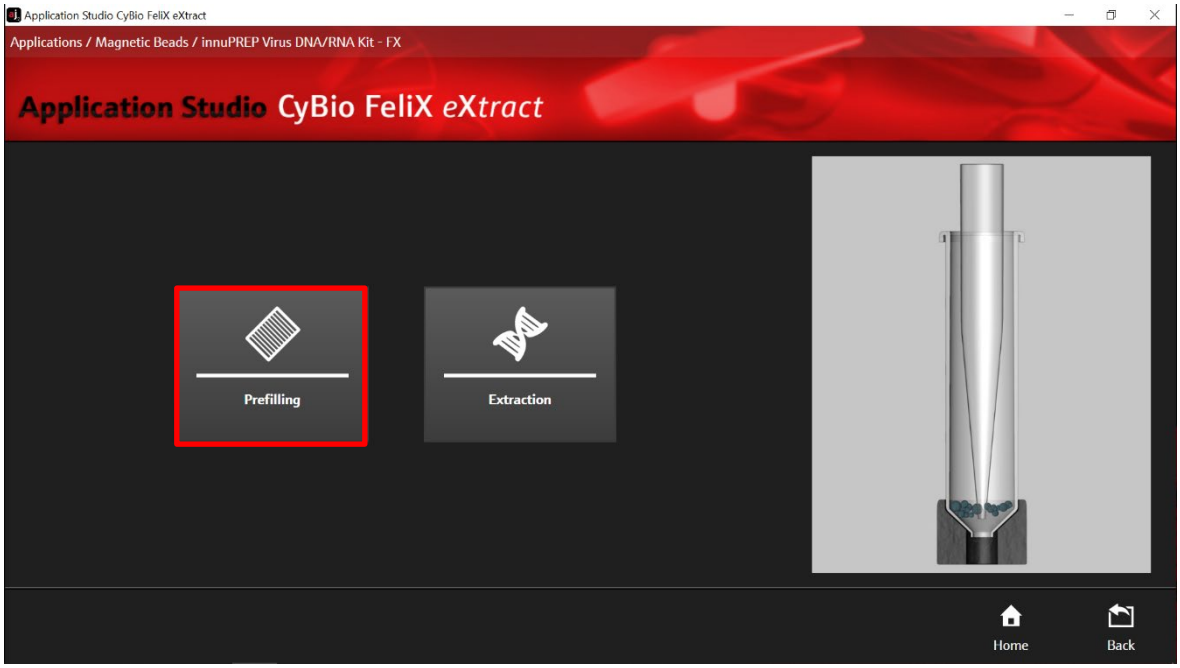


Figure 22: Routine selection in the AppStudio FeliX eXtract: Prefilling.

17. After selecting “Prefilling” the Prefilling Start Screen appears.
18. Check the correct version number of the protocol (→ see Figure 23): “Prefilling – innuPREP Virus DNA/RNA – 02”.


**Figure 23: Version number of the prefilling protocol.**

19. To adjust the sample volume, click the button “Sample Volume”. Choose “400 µL” and confirm with “Execute” (→ see Figure 24).

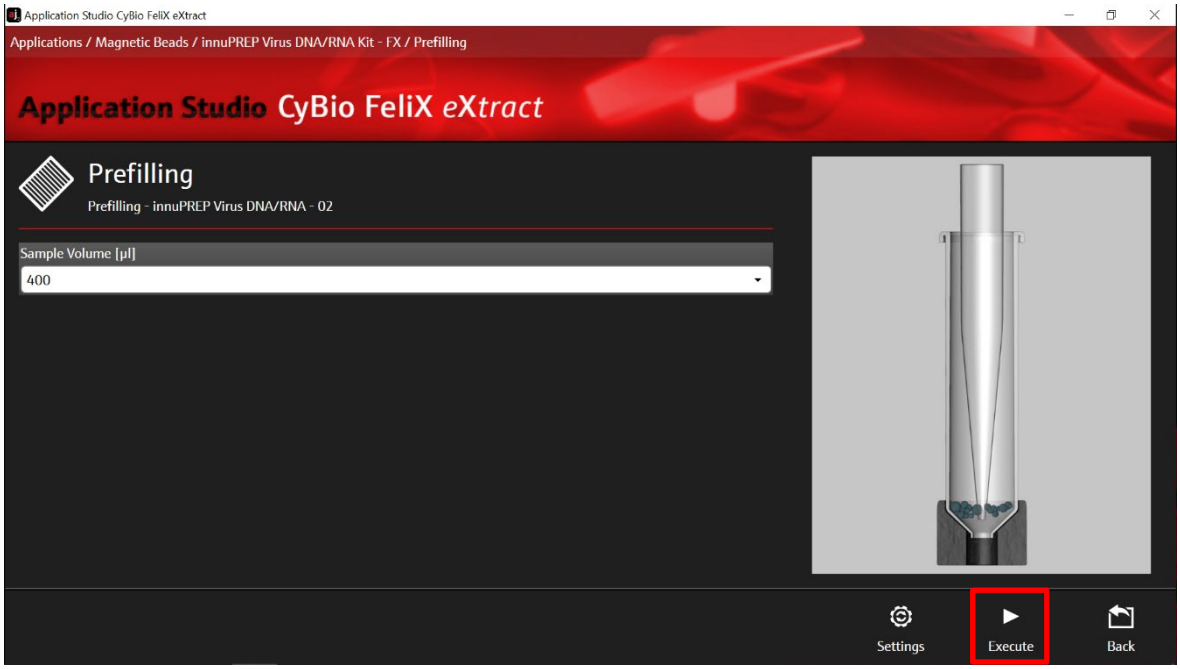


**Figure 24: Selection of sample volume: 400 µL.**


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

20. Check the correct deck position of all plates, reservoirs and hardware components (compare with list displayed in AppStudio FeliX *eXtract*, → see Figure 25) and confirm the message with "Ok".

21. 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 26).


**Figure 26: Prefilling completed.**

22. Remove the CyBio TipRack 96/1000 µL and discard all tips.
23. Remove 8-channel adapter (Head R 96) with Support 37 mm.
24. Discard the reservoirs and their contents.
25. Remove **Plate 1 – Sample / Lysis** from position 10 to prepare the plate for extraction (→ see section 14.3 “Preparing the Process Plate & Sample / Lysis Plate”, p. 43).
26. The plates **Plate 3 – Binding Solution V, Plate 4- Washing Buffer A, Plate 5 – Washing Buffer B2, Plate 2 – Process** and **the Gripper on Support 37 mm** do not have to be removed for the extraction process.
27. **Plate 7 – RNase free Water (for Elution)** has to be moved from position 1 to position 6.

## 13.2 Manual prefilling for 400 µL sample volume

Please label and prepare the following plates (→ see section 7.2 “Components not included”, p. 10) according to the table below.

| Plate | Label | Content per Well |
|---|---|---|
| **Plate 1*** | Sample / Lysis | empty (→ see section 14.2 “Sample preparation (400 µL)”, p. 42) |
| **Plate 2** | Process | **450 µL** Binding Solution V |
| **Plate 3** | Binding Solution V | **450 µL** Binding Solution V |
| **Plate 4** | Washing Solution A | **1100 µL** Washing Solution A |
| **Plate 5** | Washing Solution B2 | **1100 µL** Washing Solution B2 |
| **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.

# 14 Protocols for isolation of viral nucleic acids from 400 µL sample volume

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

## 14.1 Preparing Lysis Solution V / Carrier-Mix

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

| Component | 96 samples |
|---|---|
| **Lysis Solution V** | 52 mL |
| **Carrier Mix** | 1.3 mL |
| **Final volume** | 53.3 mL |

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

## 14.2 Sample preparation (400 µL)

**NOTE**
For the extraction of nucleic acids from swab samples we recommend the addition of Carrier Mix. Make sure that the **Carrier Mix** has been prepared as described (→ see section 9 ”Initial steps before starting”, p. 12). Ensure also that the **Lysis Solution / Carrier Mix** has been prepared according the instructions given. Lysis of the sample material is done automatically and is included in the CyBio FeliX extraction protocol.

1. **Swabs stored in a storage solution/transport medium**
   Shake the swab vigorously within the solution in the storage tube, squeeze it as completely as possible against the wall of the tube and remove the swab.
   Proceed with **400 µL of the particle-free sample** for further steps (→ see section 14.3 Preparing the Process Plate & Sample / Lysis Plate, p. 43).

2. **Dry swabs**
   Place the swabs into 1.5 mL reaction tubes containing 500 µL physiological saline (0.9 % NaCl, not included in the kit) and incubate while shaking continuously for 20 min.
   Squeeze all liquid from the swab and remove the it from the reaction tube.
   Proceed with **400 µL of the particle-free sample** for further steps (→ see section 14.3 Preparing the Process Plate & Sample / Lysis Plate, p. 43).

3. **Plasma/serum samples**
   Proceed with **400 µL of the particle-free sample** for further steps (→ see section 14.3 Preparing the Process Plate & Sample / Lysis Plate, p.43).

## 14.3 Preparing the Process Plate & Sample / Lysis Plate

**NOTE**
Steps 1 and 2 do 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). Renewed 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 **400 µL** of the **Lysis Solution V / Carrier Mix** into "**Plate 1 – Sample / Lysis"**.
3. Add **400 µL sample** into the desired cavity of the prefilled plate "**Plate 1 – Sample / Lysis"**. Please adhere to your sample layout.
4. Add **50 µL Proteinase K** into the prefilled cavities of plate "**Plate 1 – Sample / Lysis"**.

**NOTE**
The sample will be processed using the CyBio FeliX. Please follow the instructions of section 14.4 "Loading of CyBio FeliX", p. 44.

## 14.4 Loading of CyBio FeliX

1. Load all plates and accessories according to the scheme below (→ see Figure 27).

   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**
For 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**, p. 15).

**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**. Put 96 Filter Tips (or the number of tips required) into the CyBio **RoboTipTray 1-96/1000 µL** and put it on the **97 mm support**. Take care that every Filter Tip fits into a cavity of the Protective Plate.

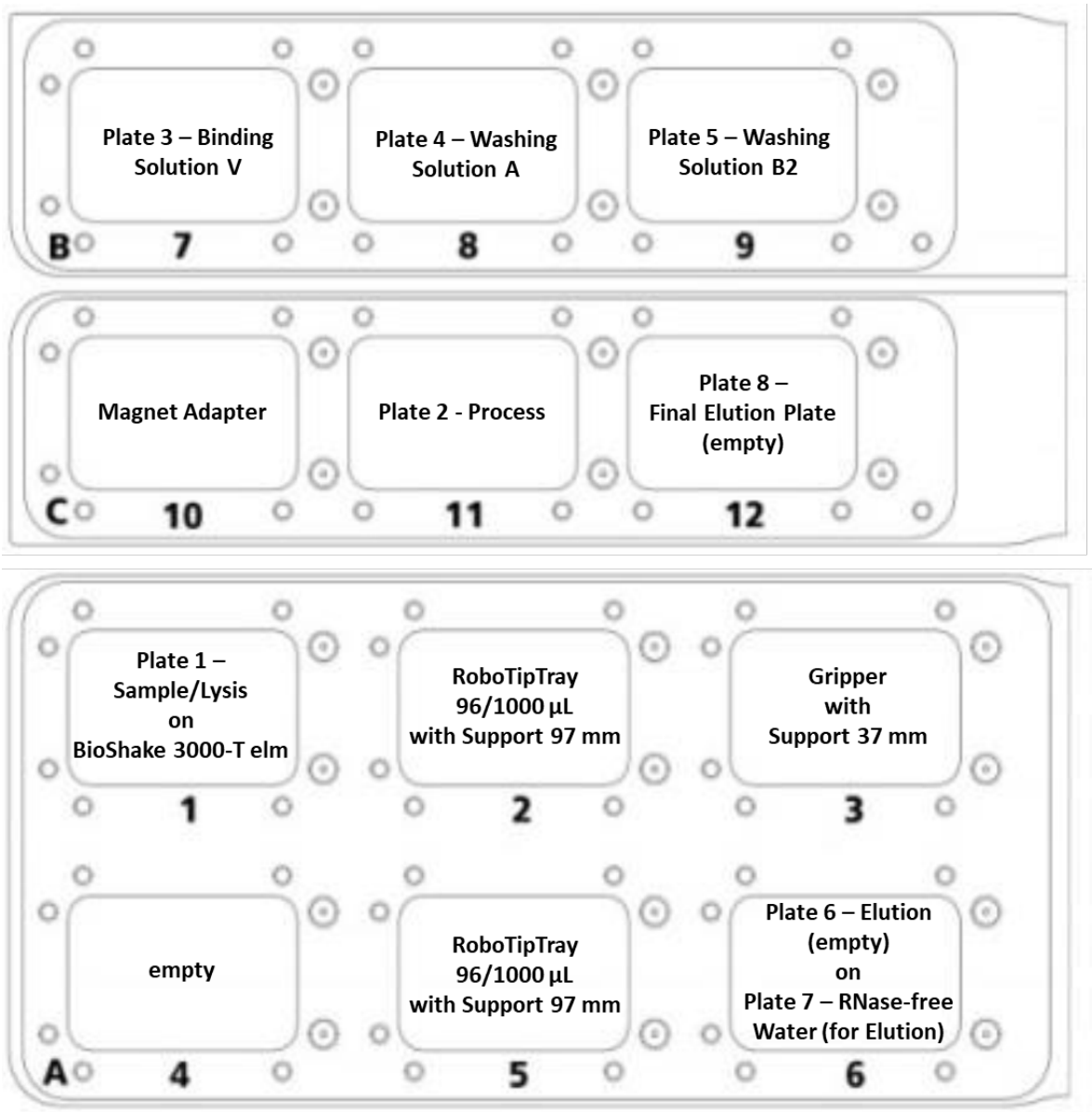


**Figure 27: Deck layout for extraction from a 400 µL sample.**

2. Switch on the CyBio FeliX and open the AppStudio FeliX *eXtract*.

3. Select “Magnetic Beads” (→ see Figure 28).

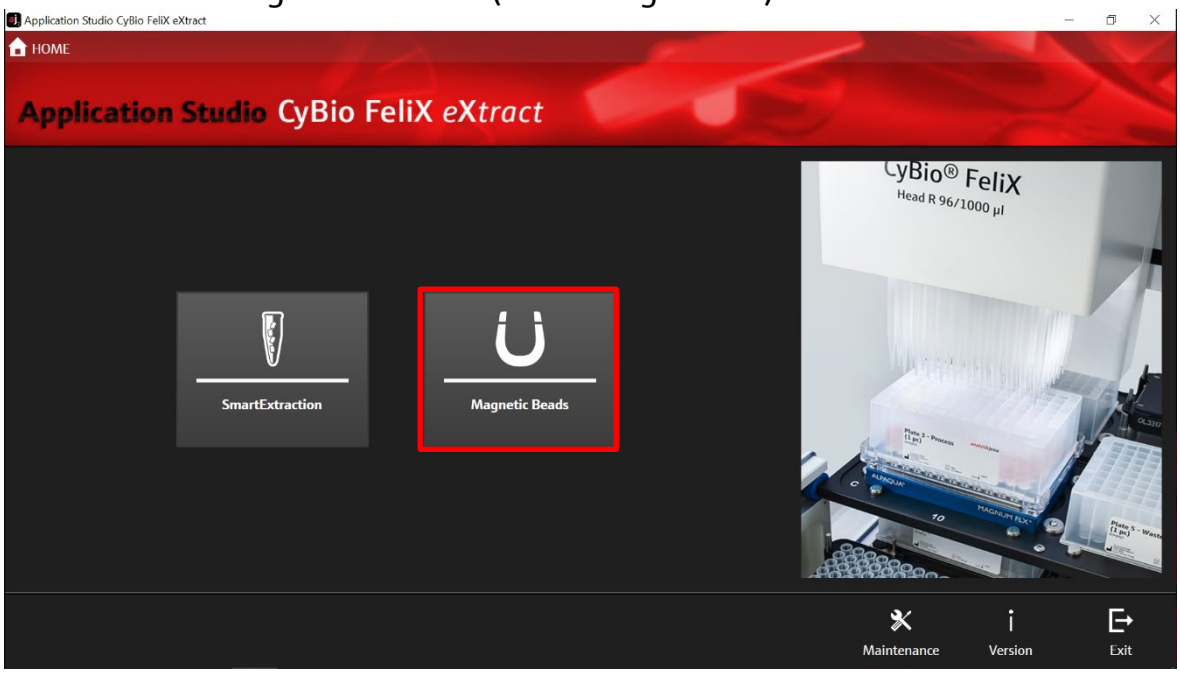


Figure 28: Homescreen of the AppStudio FeliX eXtract. Selection of Magnetic Beads

4. Select “innuPREP Virus DNA/RNA Kit – FX” (→ see Figure 29).


Figure 29: Kit selection: innuPREP Virus DNA/RNA Kit – FX.

5. Select “Extraction” (→ see Figure 30).

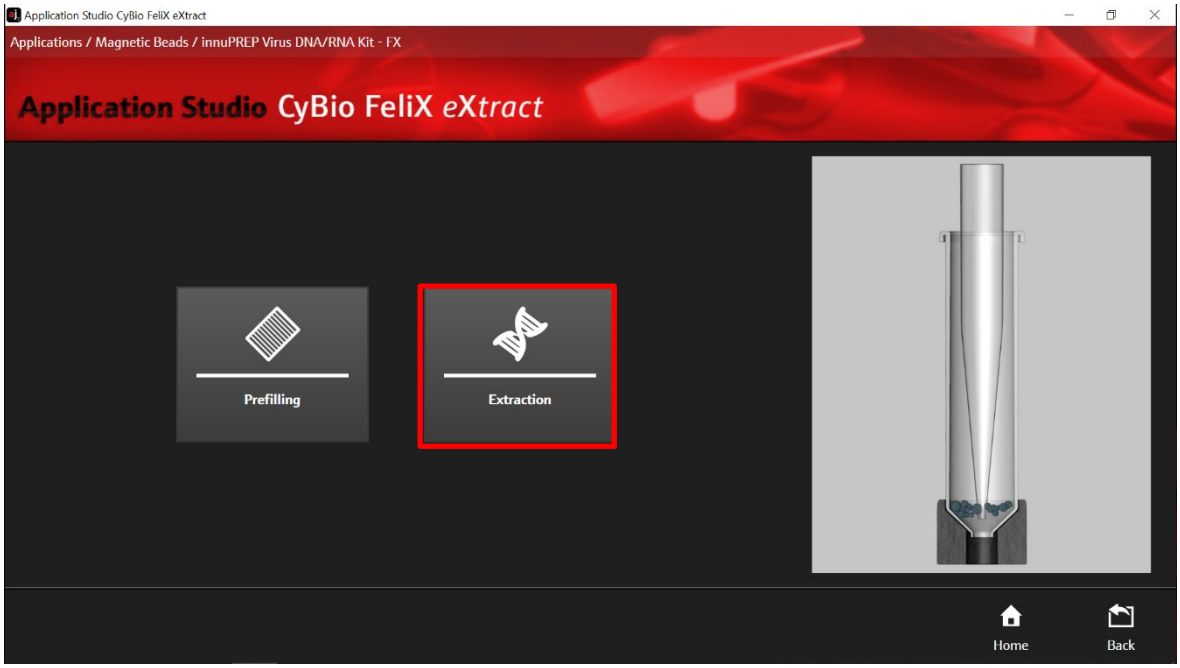


**Figure 30: Routine selection: Extraction**

6. After selecting “Extraction” the Extraction Start Screen appears (→ see Figure 32).
7. Check the correct version number of the protocol (→ see Figure 31): “Extraction - innuPREP Virus DNA/RNA – 02”.


**Figure 31: Version number of the extraction protocol**

8. Select the protocol by choosing the corresponding sample volume (400 µL) and adjust elution volume (between 50–200 µL, 100 µL are recommended) (→ see Figure 32).

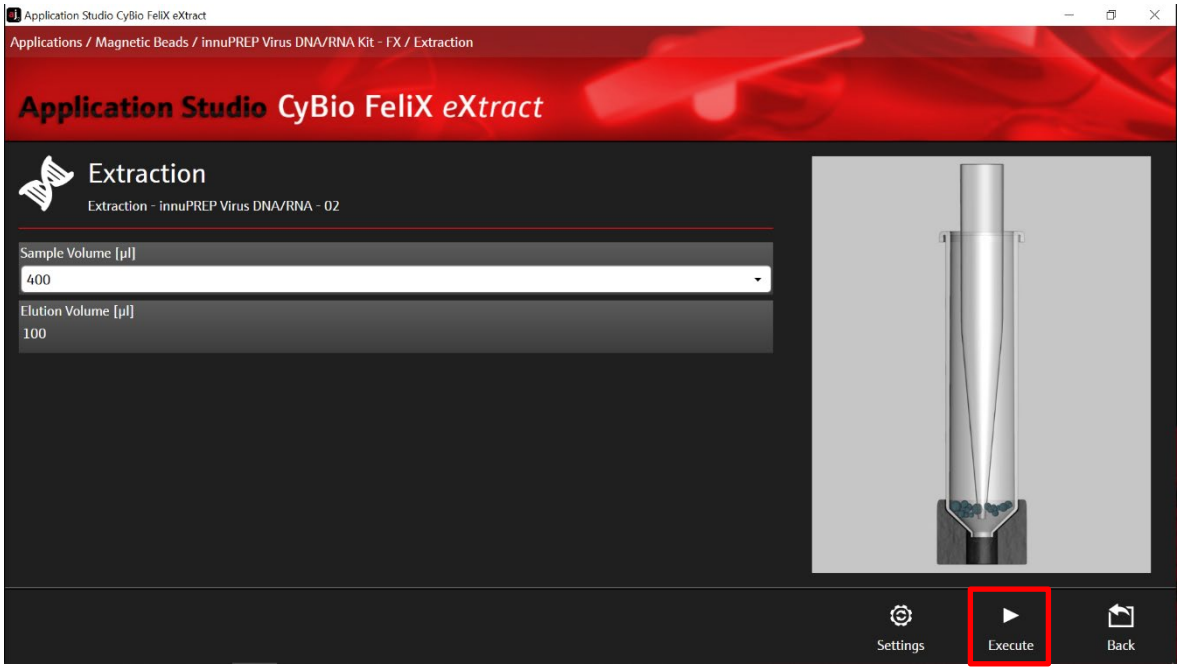


Figure 32: **Selection of sample volume (400 µL) and elution volume (variable).**

9. After selecting “Execute” the deck layout is shown. Check the correct positions of plates and accessories and confirm the message with “Ok” to start the protocol (→ see Figure 33).


**Figure 33: Deck layout for the final hardware check for the extraction.**

10. The chosen extraction protocol is performed by the CyBio FeliX.

11. Confirm the “Process completed” message with “Ok” (→ see **Figure 34**).

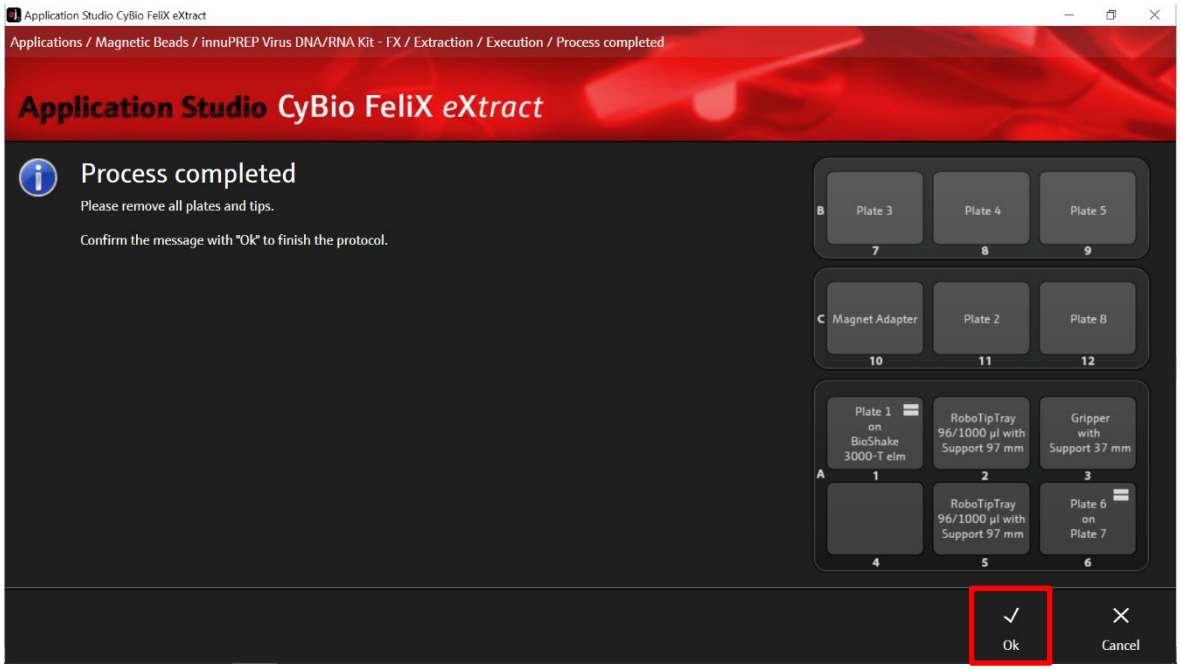


**Figure 34: Process completed.**

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

**NOTE**

When using alternate elution vessels as listed in section 14.4 “Loading of CyBio FeliX” (→ see p. 44), 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 a storage temperature of -80 °C.

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

# 15 Troubleshooting

| Problem / probable cause | Comments and suggestions |
|---|---|
| **Low amount of extracted viral DNA/RNA** | |
| Content of viral nucleic acid in sample insufficient. | Use higher sample volume, e.g. use 400 µL instead of 200 µL sample. Ensure to choose the appropriate extraction protocol. |
| Insufficient lysis of starting material. | Ensure that the required volume of 50 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. 12).<br><br>Use Internal Controls for verification of the extraction procedure. |

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