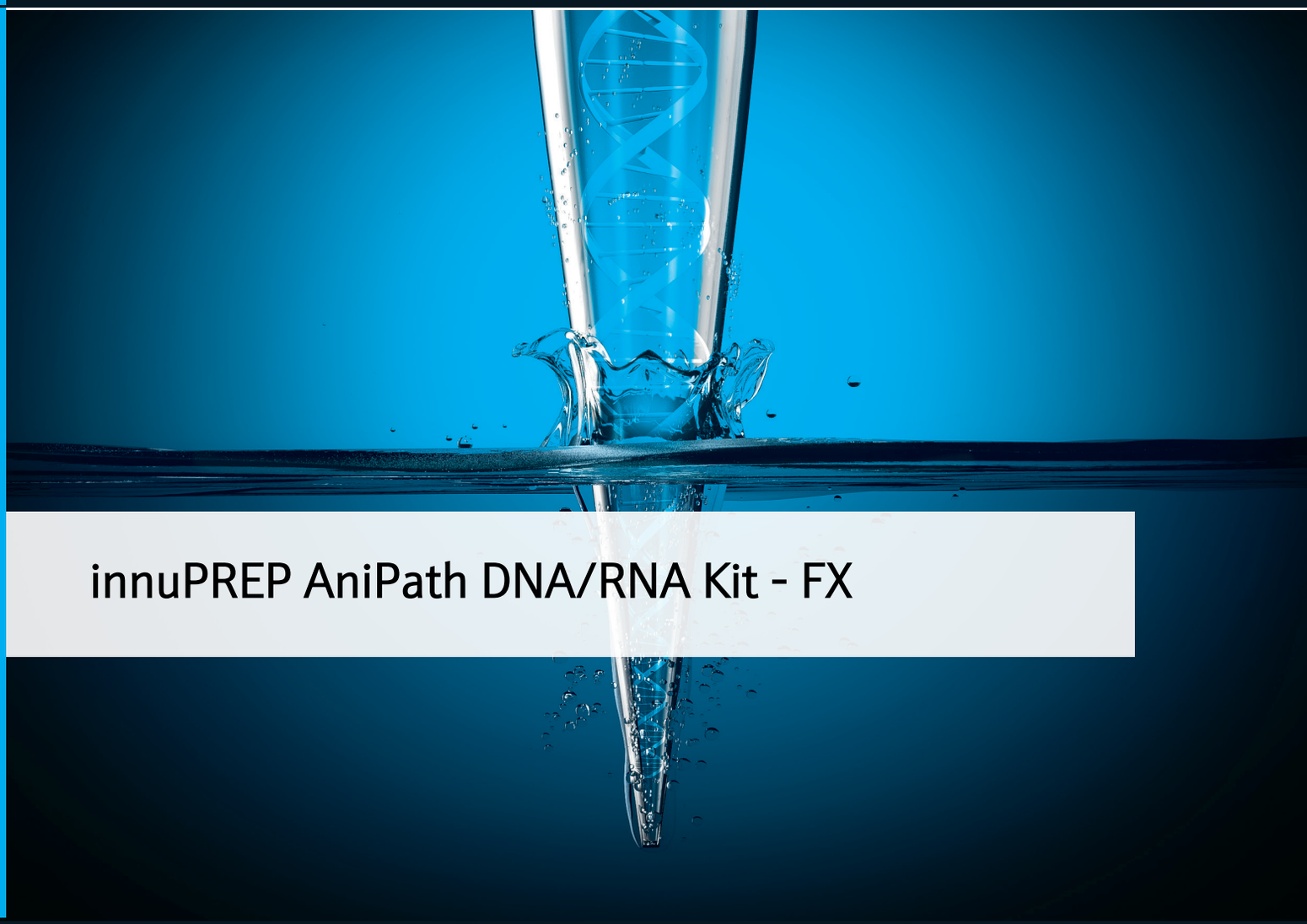
Instructions for Use
Life Science Kits & Assays



innuPREP AniPath DNA/RNA Kit - FX

Innuscreen GmbH

Order No.:
845-FX-2396096 96 reactions
845-FX-2396480 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 AniPath DNA/RNA Kit – FX** has been designed for automated isolation of viral or bacterial DNA and RNA from different kinds of starting material, like cell-free body fluids, cell culture supernatants or whole blood. Furthermore, fecal samples, swabs and other relevant starting materials can be used for isolation. The extraction procedure is based on a new patented chemistry. The kit is designed to be handled by educated personnel in a laboratory environment.

For the liquid samples, all steps of the extraction process are fully automated and run completely on the CyBio FeliX. The extraction process is based on binding of DNA and 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.

Furthermore, the kit contains a Carrier Mix comprised of a Carrier RNA and an Internal Control DNA (IC DNA) for controlling the extraction process and for better recovery of minute amounts of sample DNA. The IC DNA can be detected by real-time PCR with a corresponding real-time PCR detection kit.

Please note, the eluates will contain both, sample DNA 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 may thus be carefully optimized depending on the individual PCR system used.

CONSULT INSTRUCTIONS FOR USE
This package insert must be read carefully prior to 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. 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 when 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

The kit is shipped at ambient temperature.

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

Store 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!

All other components of the **innuPREP Anipath 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.

For further information see table “Kit components” (→ p. 10).

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 AniPath DNA/RNA Kit – 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 in the manual (→·"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 when using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA '88 regulations in the U.S. or equivalent regulations required in other countries.

All products sold by the IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
For research use only!

7. Kit components

7.1 Included kit components

	Σ 96	Σ 480
REF	845-FX-2396096	845-FX-2396480
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	5 x 2 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 Plates (square, 2.0 mL)	7	35
Final Elution Plate (1.2 mL)	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 in the kit

- 96–99.8 % ethanol

NOTE

Use only absolute/pure ethanol, NO methylated or denatured alcohol!

- 2 column and 12 column reservoirs for prefilling using CyBio FeliX (included in innuPREP Prefilling Set, OL3317-25-127)

NOTE
Only use disposable tips and plates included and recommended in the kits. The usage of other tips, reservoirs and plates may cause severe damage of the CyBio FeliX and a loss of guarantee.

Also, the usage of other components may cause malfunction of the whole protocol and loss of samples!

7.3 Required CyBio FeliX components

- CyBio FeliX Basic Unit with Enclosure (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)
- Final Elution Plate (96 well, 1.2 mL) (31-01642, 5 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)

8 Product specifications

1. Starting material:

   - Cell-free body fluids and cell culture supernatants (e.g. serum, plasma, cerebrospinal fluid; up to 400 µL)
   - Whole blood samples (up to 400 µL)
   - Swabs from nasopharyngeal samples (e.g. Influenza testing)
   - Tissue samples (up to 10 mg)
   - Stool samples (50–100 mg)

2. Processing time:

   Time required for external steps depends on the starting material

Sample volume	Automated prefilling	Extraction	Elution volume
400 µL	60 min	86 min	50–200 µL

9 Initial steps before starting

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

845-FX-2396096 845-FX-2396480	Add 1.5 mL ddH_2O to lyophilized Proteinase K.

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

845-FX-2396096 845-FX-2396480	Add 1.25 mL RNase-free Water to lyophilized Carrier Mix.

- Add absolute ethanol to each bottle of **Washing Solution B2 (conc.)** and mix thoroughly. Keep the bottle always firmly closed!

845-FX-2396096 845-FX-2396480	Add 72 mL ethanol to 48 mL Washing Solution B2 (conc.).

- Prepare Lysis Solution V/Carrier Mix as indicated in each protocol.
- 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 96 (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 only the accessories with the recommended supports! Usage of other supports or of no supports may cause damages of 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 and Preparation of Internal Controls

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.

If customized extraction controls are used, please add these components to the mixture of **Lysis Solution V** and **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 mixture **Lysis Solution V/Carrier Mix** at 4–8 °C for a maximum of 7 days.

11 Prefilling of Reagent Plates

There is the option to prefill the plates automatically with the CyBio FeliX (→ see section 11.1 below) or the possibility to prefill the plates manually (→ see section 11.2, p. 24).

11.1 Automated prefilling with CyBio FeliX

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

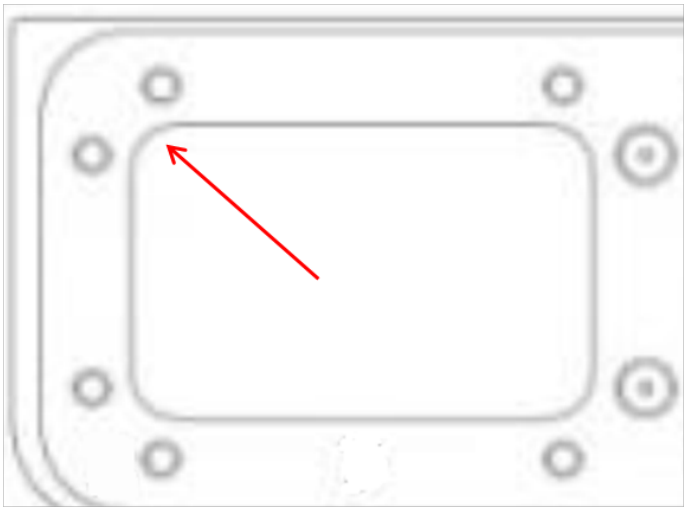

Figure 2: Positioning of plates and reservoirs on CyBio FeliX deck.

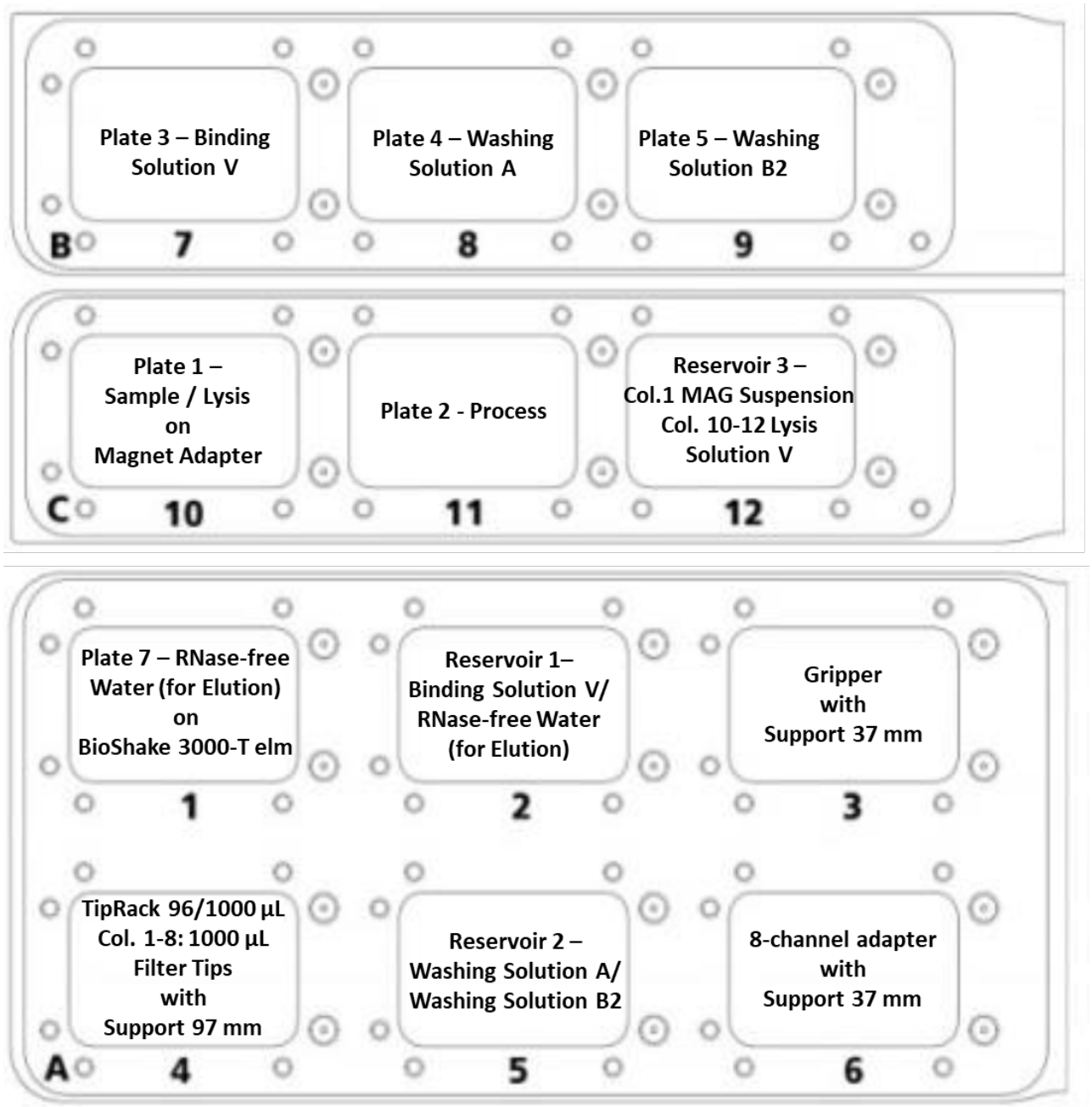

Figure 3: Deck layout for the prefilling protocol

NOTE

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

1. Label three reservoirs from the innuPREP Prefilling Set (→ see "Components not included in the kit", p. 10) according to the table below:

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

2. Label the 96 DW 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. Refer to Figure 3 (→ see p. 17) for the placement of the empty plates and the reservoirs filled as described below.
4. Transfer the content of one bottle **"Binding Solution V"** (100 mL) into the **left** side of the 2 column reservoir labeled with "Reservoir 1 - Binding Solution V/RNase-free Water (for Elution)".
5. Transfer the content of one bottle **"RNase-free Water (for Elution)"** (70 mL) into the **right** side of the 2 column reservoir labeled with "Reservoir 1 - Binding Solution V/RNase-free Water (for Elution)". Place the filled reservoir into the CyBio FeliX on position 2 (→ see Figure 3).
6. Transfer the content of one bottle **"Washing Solution A"** (120 mL) into the **left** side of the 2 column reservoir labeled with "Reservoir 2 – Washing Solution A/Washing Solution B2".
7. Transfer the content of one bottle **"Washing Solution B2"** (120 mL) into the **right** side of the 2 column reservoir labeled with "Reservoir 2 – Washing Solution A/ Washing Solution B2". Place the filled reservoir into the CyBio FeliX on position 5 (→ see Figure 3).
8. Vortex the 9 mL-bottle **MAG Suspension** properly (at least 30 s). Transfer the complete content of the bottle into column 1 of the reservoir labeled with "Reservoir 3 – MAG Suspension / Lysis Solution V".
9. Transfer 15 mL of the prepared **Lysis Solution V/Carrier Mix** (→ see "Usage and Preparation of Internal Controls", p. 14) in column 10, 15 mL in column 11 and 15 mL in column 12 of the reservoir labeled "Reservoir 3 – MAG Suspension / Lysis Solution V". Place the filled reservoir into the CyBio FeliX on position 12 (→ see Figure 3).
10. Insert Filter Tips in columns 1-8 in the CyBio TipRack 96/1000 µL.
11. Place the CyBio TipRack 96/1000 µL into the CyBio FeliX on position 4 (→ see Figure 3).
12. Place the 8-channel adapter (Head R 96) with the support 37 mm into the CyBio FeliX on position 6 (→ see Figure 3).

13. Place the empty, labeled plates on the CyBio FeliX deck according to the deck layout as shown in 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.

14. Turn on the CyBio FeliX and open the AppStudio FeliX *eXtract*.
15. Select "Magnetic Beads" (→see Figure 4).

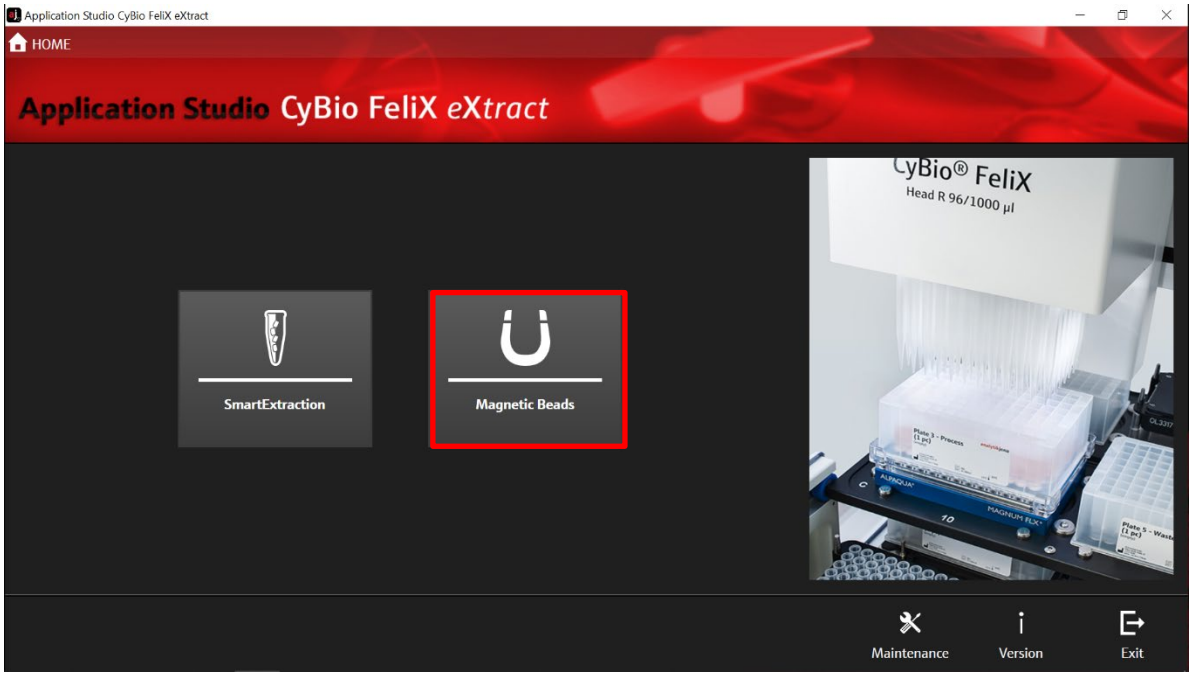

Figure 4: Home screen of the Application Studio FeliX *eXtract*.

17. Select “innuPREP AniPath DNA/RNA Kit – FX” (→ see Figure 5).

Figure 5: Kit selection in the Application Studio FeliX *eXtract*.

18. Select “Prefilling” (→ see Figure 6).

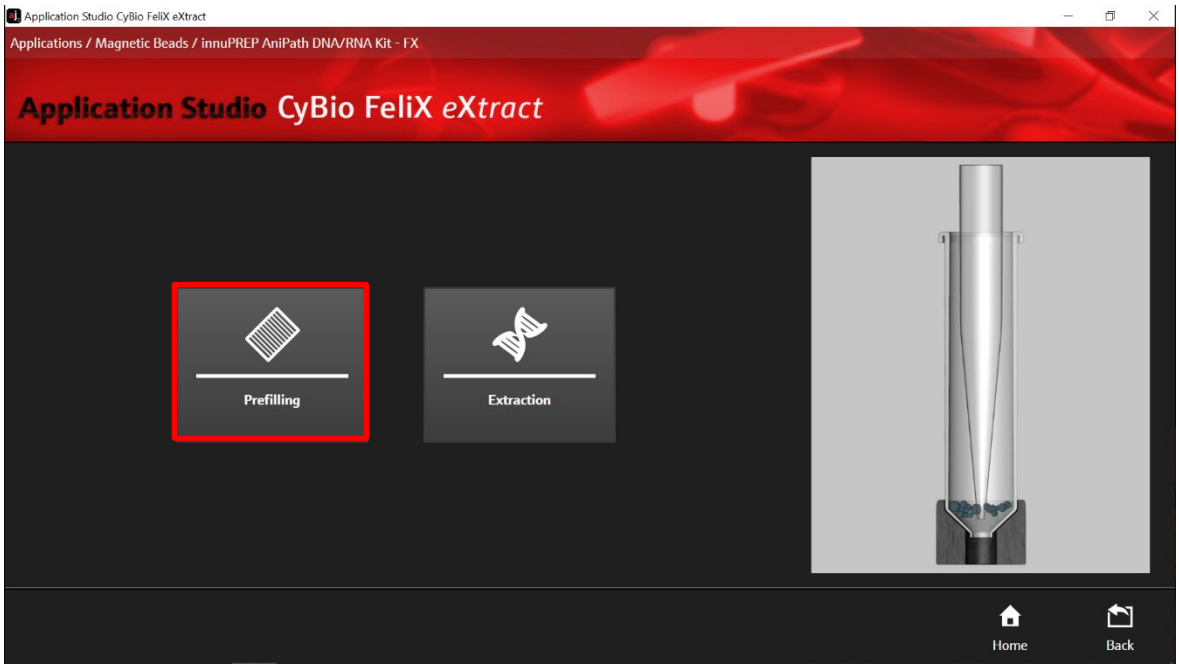

Figure 6: Routine selection in the Application Studio FeliX *eXtract:* Prefilling.

19. After selecting “Prefilling” the Prefilling-Start-Screen appears.
20. Check the correct version number of the protocol (→ see Figure 7): “Prefilling – innuPREP AniPath DNA/RNA - 01”.

Figure 7: Version number of the prefilling protocol.

21. Check the correct deck position of all plates, reservoirs and hardware components (compare with list displayed in the AppStudio FeliX e*Xtract*, → see Figure 8) and confirm the message with “Ok”.

Figure 8: Deck layout in the AppStudio FeliX *eXtract*.

22. 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 9: Prefilling completed.

23. Remove the CyBio TipRack 96/1000 µL and discard all tips.
24. Remove 8-channel adapter (Head R 96) with Support 37 mm.
25. Dispose of the reservoirs and all their contents.
26. The plates **Plate 3 – Binding Solution V, Plate 4 - Washing Solution A, Plate 5 – Washing Solution 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.
28. Remove Plate 1 – Sample/Lysis from the deck and proceed with ”Protocols for isolation of nucleic acids” on p. 25.

11.2 Manual prefilling

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

Plate	Label	Content per Well
Plate 1*	Sample/Lysis	empty (→ see “Usage and Preparation of Internal Controls”, p.14)
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 should be prefilled.

12 Protocols for isolation of nucleic acids

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 Protocol I: Isolation of viral or bacterial nucleic acids from up to 400 µL cell-free body fluids (serum, plasma, cerebrospinal fluid), cell culture supernatants and whole blood

NOTE
When using cell free body fluids, we recommend the addition of Carrier Mix.
Ensure that the Carrier Mix has been prepared as described (→ see "Initial steps before starting", p. 12; "Usage and Preparation of Internal Controls", p. 14).
Lysis of the sample material is done automatically and is included in the CyBio FeliX extraction protocol.

NOTE
Steps 1 and 2 do not have to be done when the plates were prefilled with the CyBio FeliX.

NOTE
It is important to mix the **MAG Suspension** by vigorous shaking or vortexing before use (approx. 30 s). Repeated vortexing after pipetting 10 samples 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 cavities of the prefilled plate "**Plate 1 – Sample/Lysis"**. Please adhere to your sample layout.
4. Add **50 µL Proteinase K** to the prefilled cavities of **"Plate 1 – Sample/Lysis"**.

NOTE

If the volume of the blood sample is less than 400 µL, fill up to 400 µL using PBS.

NOTE

The sample will be processed using the CyBio FeliX. Please follow the instructions of section 13, "Automated extraction using the CyBio FeliX".

12.2 Protocol II: Isolation of viral nucleic acids from swabs from nasopharyngal samples (e.g. for Influenza testing)

NOTE

For the extraction of nucleic acids from swab samples we recommend the addition of Carrier Mix.
Ensure that the Carrier Mix has been prepared as described (→ see "Initial steps before starting", p. 12; "Usage and Preparation of Internal Controls", p. 14).

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

NOTE

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

NOTE

Steps 1 and 2 do not have to be done when the plates were prefilled with the CyBio FeliX.

1. Transfer **50 µL of MAG Suspension** directly into the liquid of the prefilled plate "**Plate 2 – Process**".
2. Transfer **400 µL Lysis Solution V/Carrier Mix** directly into the plate **"Plate 1 – Sample/Lysis"**.
3. Place the swabs into 1.5 mL reaction tubes containing **500 µL physiological saline** (0.9 % NaCl, not included in the kit) and incubate under continuous shaking for 20 min
4. Squeeze the swab and remove it from the reaction tube.
5. Add **400 µL** of the **liquid sample** into the desired cavities of the prefilled plate "**Plate 1 – Sample/Lysis"**. Please adhere to your sample layout.

6. Add **50 µL Proteinase K** to the prefilled cavities of **"Plate 1 – Sample/Lysis"**.

NOTE
The sample will be processed using the CyBio FeliX. Please follow the instructions of section 13, p. 33 "Automated extraction using the CyBio FeliX".

12.3 Protocol III: Isolation of viral nucleic acids from tissue homogenates

NOTE
Co-extraction of genomic nucleic acids can inhibit downstream PCR or real-time PCR applications!

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

NOTE
Steps 1 and 2 do not have to be done when the plates were prefilled with the CyBio FeliX.

1. Transfer **50 µL of MAG Suspension** directly into the liquid of the prefilled plate "**Plate 2 – Process**".
2. Transfer **400 µL Lysis Solution V/Carrier Mix** directly into the plate **"Plate 1 – Sample/Lysis"**.
3. Homogenize the tissue samples using bead-based homogenizers (e.g. SpeedMill PLUS, Analytik Jena GmbH). For optimized results use 5-10 mg of tissue sample.

   Transfer the tissue sample into a homogenization tube and add **600 µL RNase-free water** or PBS.
4. After homogenization centrifuge the sample at 10,000 x g for 2 min.
5. Add **400 µL of the homogenized tissue** sample to the prefilled cavities of the plate **"Plate 1 –Sample/Lysis"**.
6. Add **50 µL Proteinase K** to the prefilled cavities on **"Plate 1 – Sample/Lysis"**.

NOTE
The sample will be processed using the CyBio FeliX. Please follow the instructions of section 13, p. 33 “Automated extraction using the CyBio FeliX”.

12.4 Protocol IV: Isolation of nucleic acids from stool samples (e.g. Norovirus extraction)

NOTE
In some cases, the initial fecal sample is mixed with special buffers for subsequent ELISA detection of different viruses. In this case use Option 2.

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

NOTE
Steps 1 and 2 do not have to be done when the plates where prefilled with the CyBio FeliX.

Option 1: Standard procedure

1. Transfer **50 µL of MAG Suspension** directly into the liquid of each cavity the prefilled plate “**Plate 2 – Process”**.
2. Transfer **400 µL** of the **Lysis Solution V/Carrier Mix** into the plate “**Plate 1 – Sample/Lysis”**.
3. Transfer 50–100 mg stool sample into a 1.5 mL reaction tube.
4. Add **250 µL PBS** (not included in the kit). Vortex the tube for 10 sec.
5. Centrifuge the tube at maximum speed for 3 min.

6. Add **200 µL** of the clear supernatant into the desired cavity of the prefilled plate "**Plate 1 – Sample/Lysis"**. Please adhere to your sample layout plan.
7. Add **200 µL PBS** into the prefilled cavities of the plate "**Plate 1 – Sample/Lysis"**.
8. Add **50 µL Proteinase K** into the prefilled cavities of the plate "**Plate 1 – Sample/Lysis"**.

NOTE
The sample will be processed using the CyBio FeliX. Please follow the instructions of section 13, p. 33 "Automated extraction using the CyBio FeliX".

Option 2: Fecal sample is already mixed with ELISA Buffer

NOTE
Steps 1 and 2 do not have to be done when the plates where prefilled with the CyBio FeliX.

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. Use **250 µL** of the sample, transfer it into a 1.5 mL reaction tube and centrifuge the tube at maximum speed for 3 min.
4. Add **200 µL** of the clear supernatant into the desired cavities of the prefilled plate "**Plate 1 – Sample/Lysis"**. Please adhere to your sample layout.
5. Add **200 µL PBS** into the prefilled cavities of the plate "**Plate 1 – Sample/Lysis".**
6. Add **50 µL Proteinase K** into the prefilled cavities of the plate "**Plate 1 – Sample/Lysis"**.

NOTE

The sample will be processed using the CyBio FeliX. Please follow the instructions of section 13, p. 33 “Automated extraction using the CyBio FeliX”.

13 Automated extraction using the CyBio FeliX

1. Load all plates and accessories according to the scheme below (→ see Figure 10: Deck layout for extraction").
   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.
Place position A1 in the top left corner of the CyBio FeliX deck (→ see Figure 2, p. 16).

NOTE
Please pay special attention to the following deck positions:

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

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.

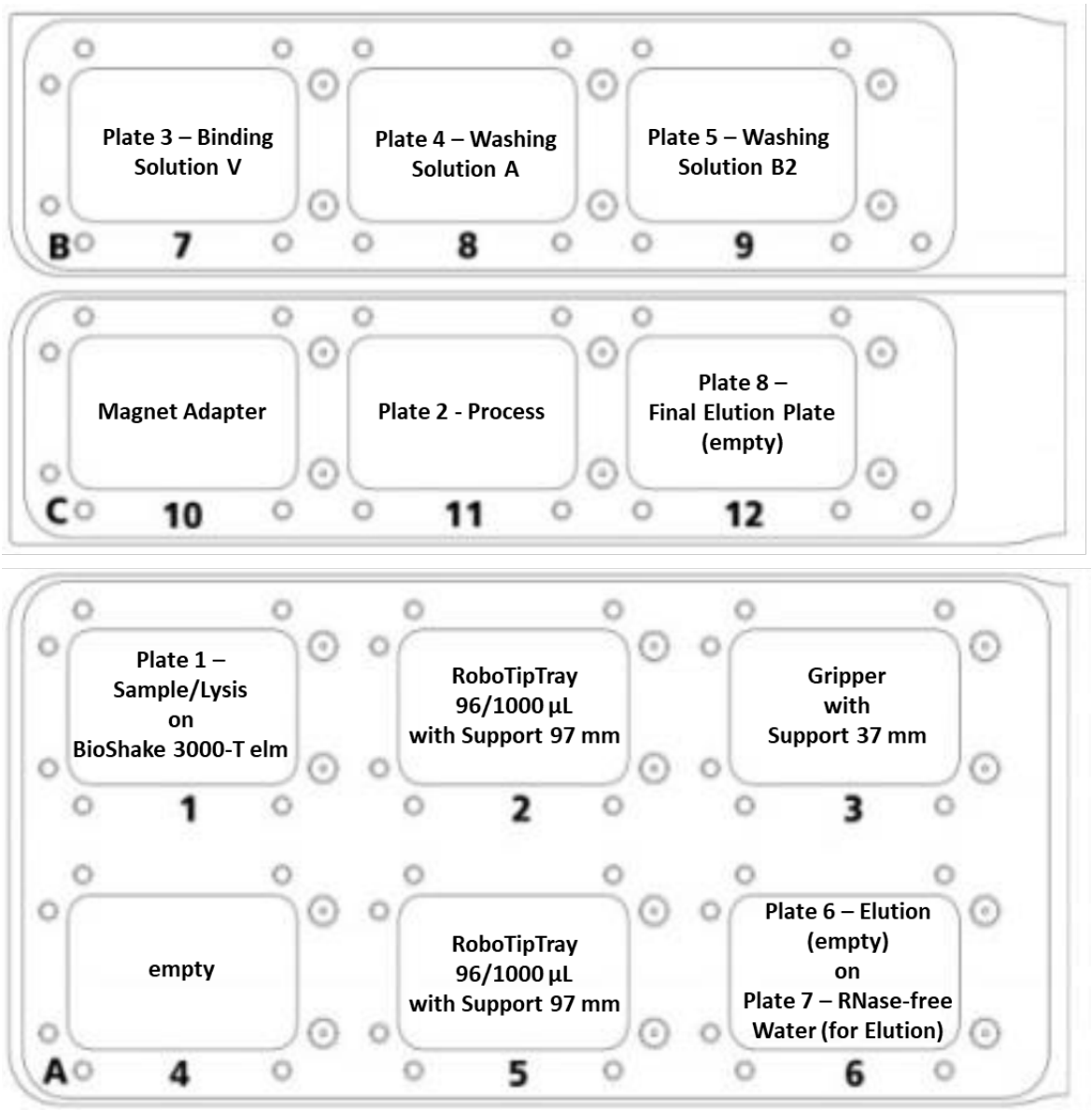

Figure 10: Deck layout for extraction

2. Turn on the CyBio FeliX.
3. Select and open the AppStudio FeliX *eXtract*.
4. Select “Magnetic Beads” (→ see Figure 11).

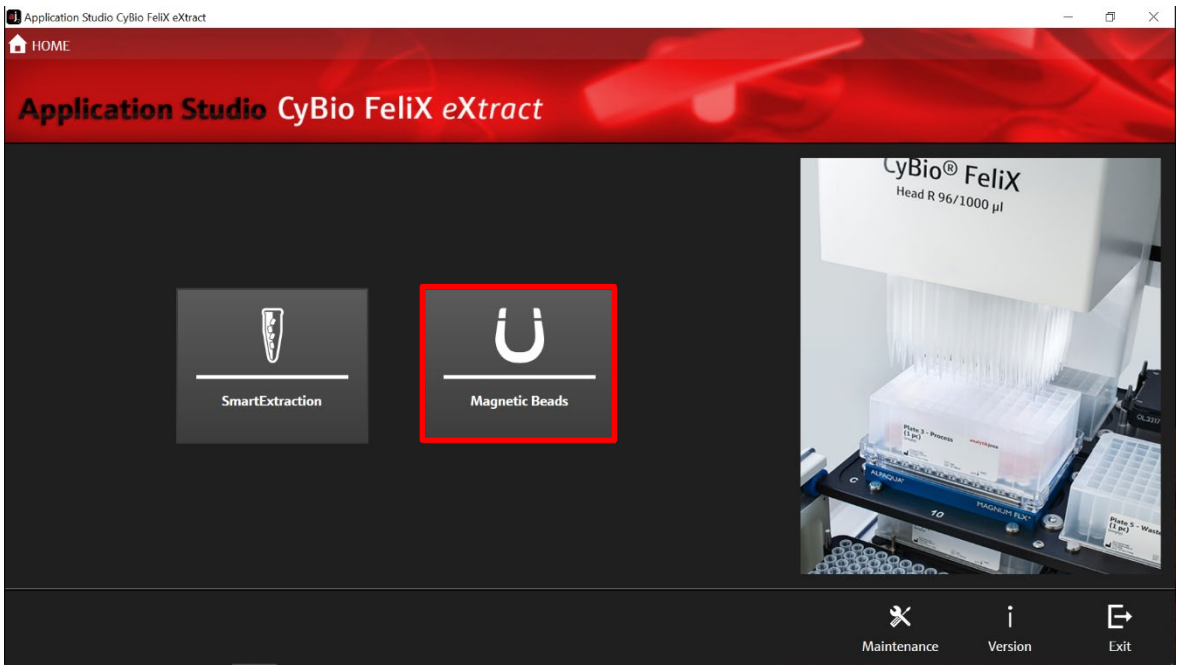

Figure 11: Screen for selecting a Magnetic Beads protocol.

5. Select “innuPREP AniPath DNA/RNA Kit – FX” (→ see Figure 12).

Figure 12: Kit selection in the AppStudio FeliX *eXtract*.

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

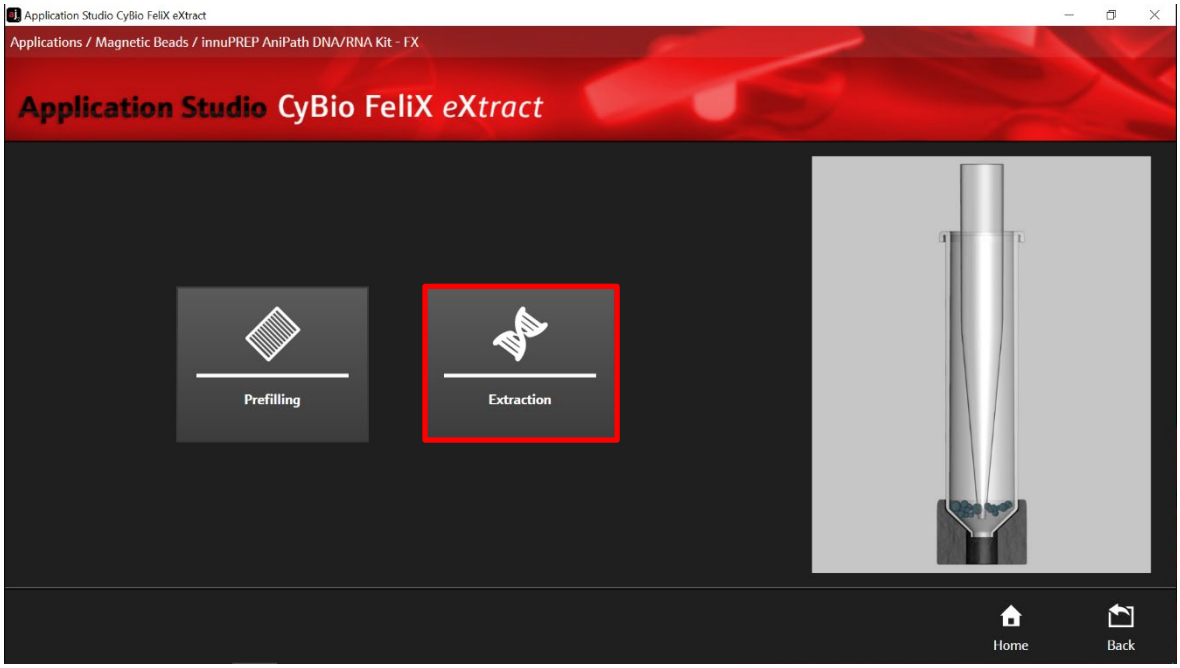

Figure 13: Routine selection in the AppStudio FeliX *eXtract*: Extraction.

7. After selecting "Extraction" the Extraction Start Screen appears (→ see Figure 15).
8. Check the correct version number of the protocol: "Extraction - innuPREP AniPath DNA/RNA – 01" (→ see Figure 14).

Figure 14: Version number of the extraction protocol.

9. Adjust the elution volume (elution volumes range from 50 µL to 200 µL (→ see Figure 15). 100 µL are recommended for general applications or to start establishing a workflow using extraction and downstream applications).

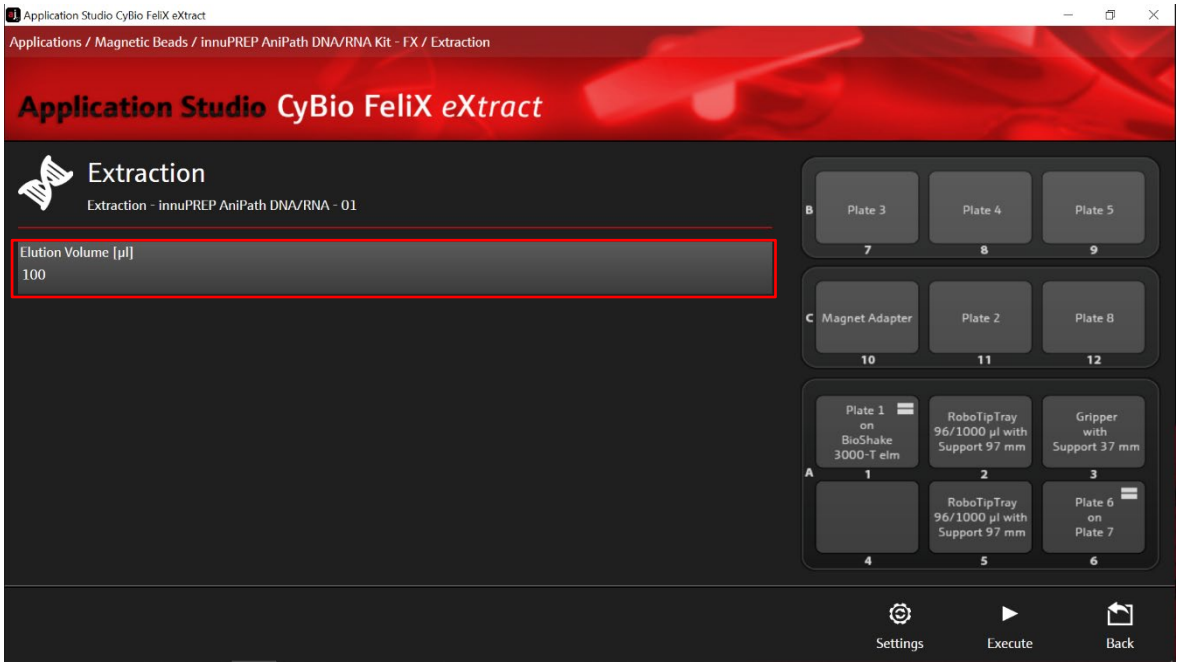

Figure 15: Adjustment of the elution volume.

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

Figure 16: Deck layout in the AppStudio FeliX *eXtract*.

Figure 17: Screen after completion of the protocol.

11. Confirm the “Process completed” message with “Ok” (→ see Figure 17).
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 13 “Automated extraction using the CyBio FeliX” (→ see p. 33), 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.

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

14 Troubleshooting

Problem/probable cause	Comments and suggestions
Low amount of extracted viral RNA/DNA	
Content of nucleic acid in sample insufficient.	Use higher sample volumes, e.g. use 400 µL instead of 200 µL sample.
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 “Product specifications” (→ see p. 12). Use Internal Controls for verification of the extraction procedure.

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