Instructions for Use
Life Science Kits & Assays

innuPREP Plant DNA Kit - FX

Innuscreen GmbH

Order No.:
845-FX-2196096 96 reactions
845-FX-2196480 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!

Manufacturer and Distributor:
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

Contents

1 Introduction

1.1 Intended use

The **innuPREP Plant DNA Kit - FX** has been designed for automated extraction of genomic DNA from plant sample materials. The extraction procedure is based on a newly patented chemistry. The kit is designed to be handled by educated personnel in a laboratory environment.

The procedure starts with the lysis of the starting material. All subsequent steps are automated and run completely on the CyBio FeliX. The extraction process is based on binding of DNA 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.

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, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling 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 Storage conditions

All kit components are shipped at ambient temperature.

Store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

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

All other components of the **innuPREP Plant 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.

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

For further information see table “Kit components” (→ p. 7).

4 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 Plant DNA 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.

5 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.9). 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!

6 Kit components

6.1 Included kit components

	Σ 96	Σ 480
REF	845-FX-2196096	845-FX-2196480
Lysis Solution CBV	50 mL	250 mL
Proteinase K	for 2 x 1.5 mL working solution	for 7 x 1.5 mL working solution
Precipitation Buffer P	2 x 6 mL	2 x 30 mL
Prefilter	2 x 50	10 x 50
Receiver Tubes	2 x 50	10 x 50
MAG Suspension F	2 x 0.25 mL	2 x 1.1 mL
Binding Solution SBS	15 mL	5 x 15 mL
Washing Solution A	40 mL	5 x 40 mL
Washing Solution B2 (conc.)	1 x 16 mL	5 x 16 mL
RNase-free Water	2 x 2 mL	2 x 25 mL
RNase-free Water (for Elution)	70 mL	5 x 70 mL
Deep Well Plate (2.0 mL)	6	5 x 6
Final Elution Plate	1	5
Sealing Foil	1	5
Filter Tips	2 x 96	10 x 96
Protective Plate	2	10
Manual	1	1

6.2 Components not included in the kit

- 96–99.8 % Ethanol (molecular biology grade, undenatured)
- 80 % Ethanol
- ddH_2O
- 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)

6.3 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)

6.4 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!

7 Product Specifications

1. Starting material:

- Disrupted plant material
- 20 – 60 mg cornmeal, seeds
- Sugar beet, corn leaf (10–40 mg fresh material / 1–4 mg dried material)

NOTE
Avoid freezing and thawing of undisrupted material. If plant tissue will not be disrupted immediately after harvesting, it can be stored in liquid nitrogen, lyophilized/dried or frozen. Fresh material can be kept at 4 °C to 8 °C for 24 h but should be frozen at -22 °C to -18 °C or for longer storage at -80 °C for later processing. Ground tissue powder can also be stored at -80 °C. Alternatively, tissue can be dried or lyophilized after harvesting to allow storage at room temperature (15 °C to 30 °C). To ensure DNA quality, samples should be completely dried within 24 h of collection.

Processing Time:

Sample volume	Automated prefilling	Extraction	Elution volume
200 µL	26 min	40 min	50–200 µL

8 Initial steps before starting

- Add 1.5 mL ddH_2O to each vial of lyophilized **Proteinase K**, mix thoroughly and store as described above.
- Add the indicated amount of absolute ethanol to **Washing Solution B2 (conc.)** and mix thoroughly. Keep the bottles always firmly closed!

845-FX-2196096	Add 24 mL ethanol (96–99.8 %) to each bottle of Washing Solution B2 (conc.).

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

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 CyBio TipRack 96/1000 µL (right).

9 Protocol for lysis of plant material

1. Add **400 µL Lysis Solution CBV** and **20 µL Proteinase K** to the **homogenized** sample (e.g. SpeedMill, Analytik Jena GmbH) and mix vigorously by pulsed vortexing for 5 s.
2. Incubate sample for 60 min at 60 °C and 1200 rpm in a shaking platform.

NOTE
To remove RNA from the sample (optional) add 1 µL of RNase A solution (10 mg/mL), vortex shortly and incubate for 10 minutes at room temperature. Be sure that the RNase A is free of DNase-activity.

3. Add **80 µL Precipitation Buffer P** and vortex the sample for 5 s. Incubate at room temperature for 5 min and centrifuge at maximum speed for 5 min.
4. Transfer the clear supernatant onto a Prefilter located in a Receiver Tube and centrifuges the tube at 11,000 x g (~11,000 rpm) for 1 min. Discard the Prefilter. The filtrate is used for automated extraction.
5. Proceed with “Prefilling of Reagent Plates”.

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 the 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**).

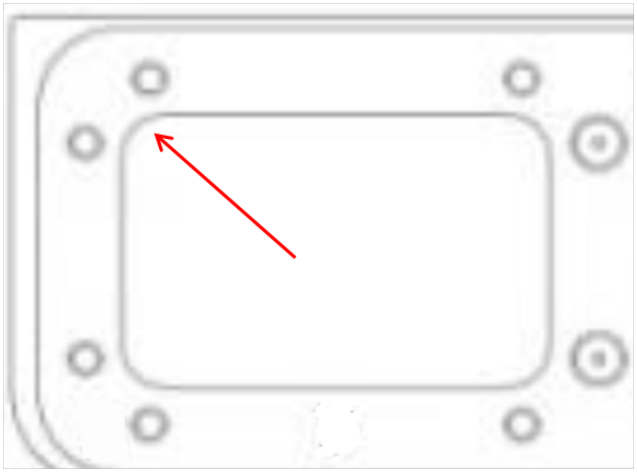

Figure 2: Positioning of plates and reservoirs on CyBio FeliX deck.

1. Label three reservoirs from the innuPREP Prefilling Set (→ see "Components not included in the kit", p. 8) according the table below:

Number	Label	
Reservoir 1 (2 column)	**Reservoir 1:** Left side of reservoir: Right side of reservoir:	 80 % EtOH 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	Binding solution SBS

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

Plate	Label
Plate 1	Process
Plate 2*	Waste (empty)
Plate 3	Washing Solution A
Plate 4	Washing Solution B2
Plate 5	80% Ethanol
Plate 6	RNase-free Water (for Elution)
Plate 7*	Final Elution Plate (empty)

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

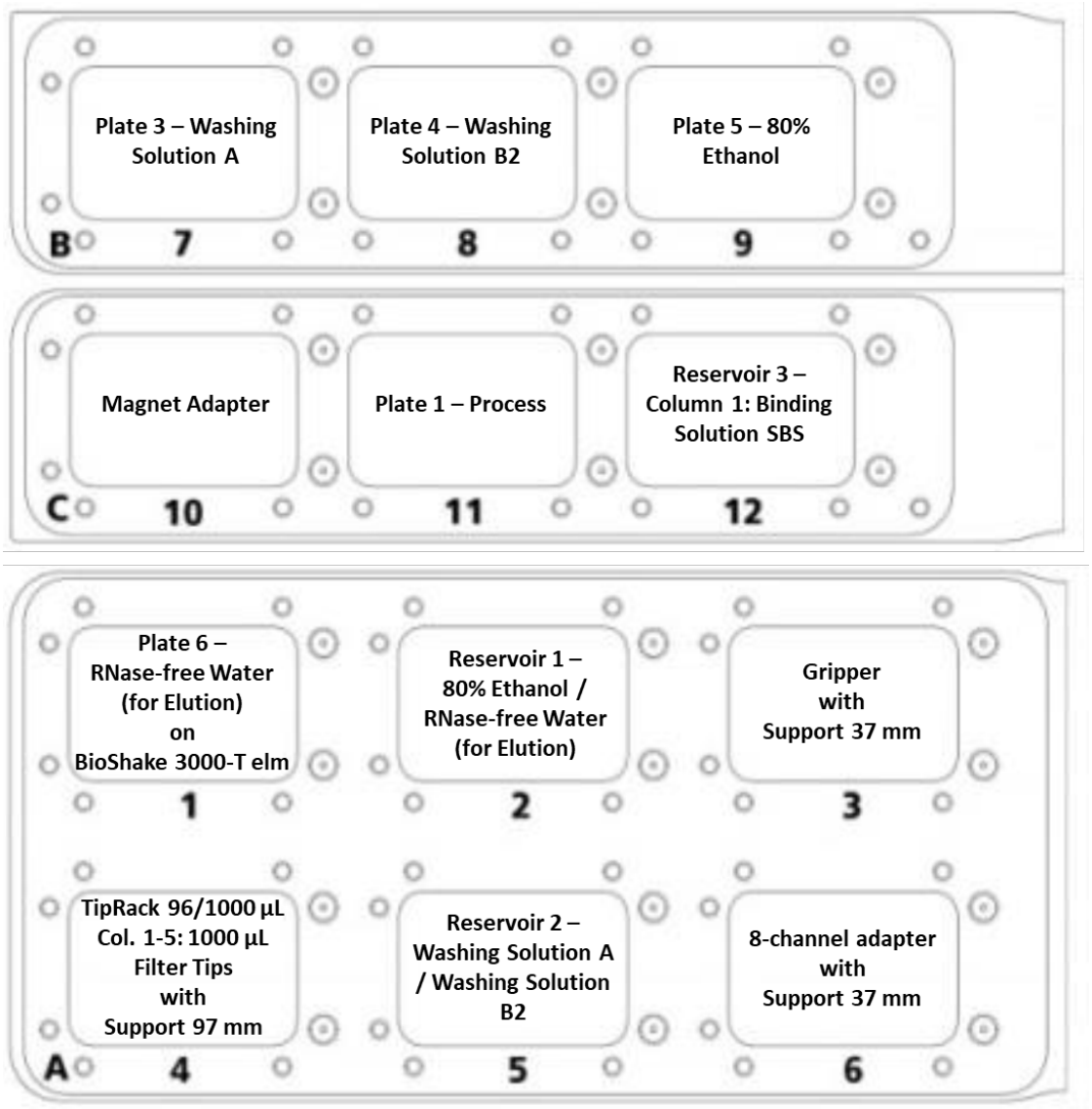

Figure 3: Deck layout for starting the prefilling protocol for 200 µL sample volume.

3. Transfer 40 mL **80 % Ethanol** into the **left** side of the 2 column reservoir labeled "Reservoir 1 – 80% Ethanol / 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 – 80% EtOH / 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 (40 mL) of **"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 (40 mL) of **"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. Transfer the content of one bottle (15 mL) of **"Binding Solution SBS"** into column A1 of the reservoir labeled "Reservoir 3 – Binding Solution SBS". Place the filled reservoir into the CyBio FeliX on position 12 (→ see **Figure 3**).
8. Insert Filter Tips in columns 1-5 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**).

NOTE
Please pay special attention to the following deck position:

Position 1:
Place **Plate 6 – RNase-free Water (for Elution)** directly onto the BioShake 3000-T elm.

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

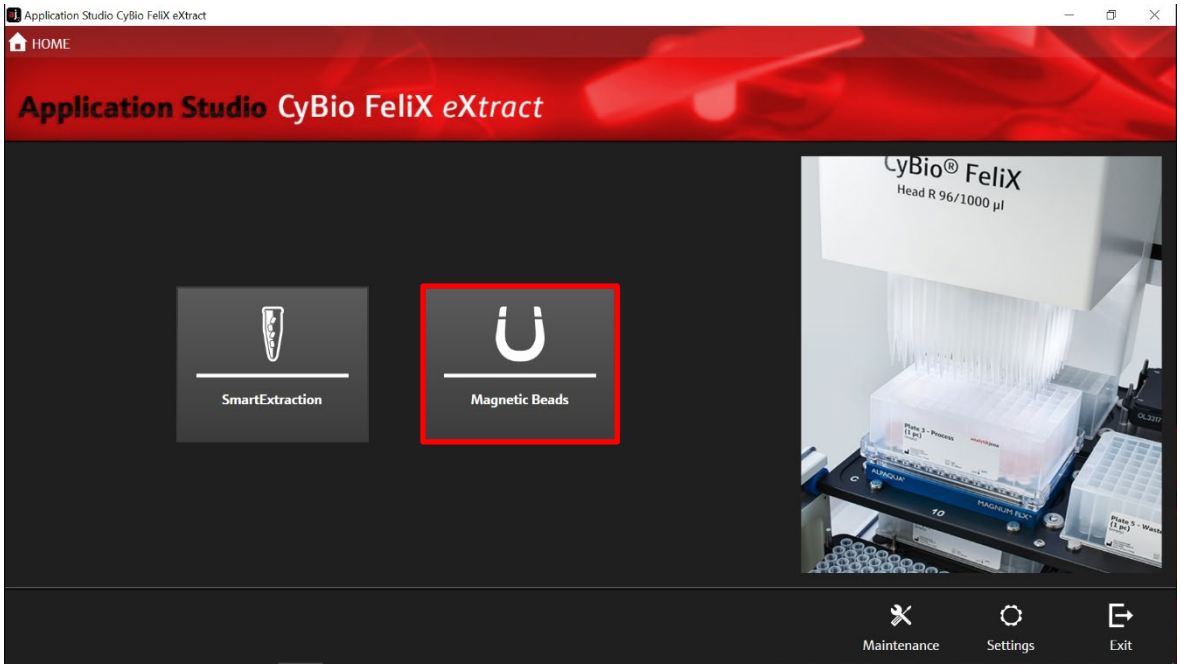

Figure 4: Homescreen of the AppStudio FeliX eXtract.

14. Choose “innuPREP Plant DNA Kit – FX” (→ see

15. **Figure 5**).

Figure 5: Kit selection in the AppStudio FeliX eXtract.

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

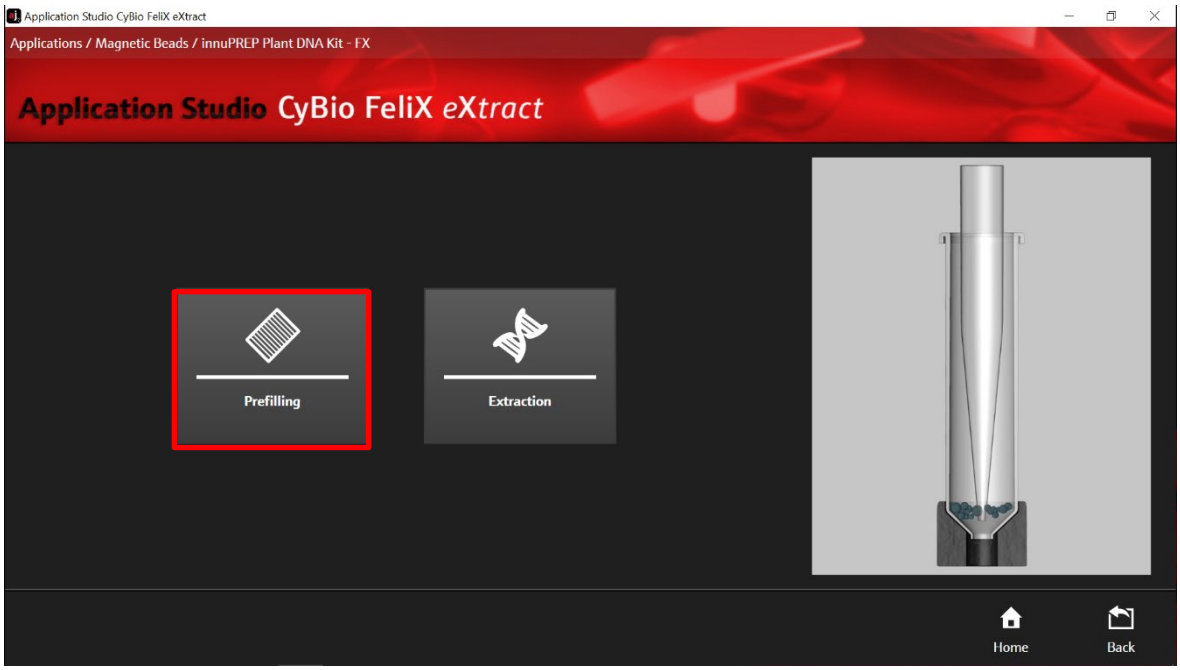

Figure 6: Routine selection in the AppStudio FeliX eXtract: Prefilling.

17. After choosing “Prefilling” the Prefilling Start Screen appears.
18. Check the correct version number of the protocol (→ see **Figure 7**): “Prefilling – innuPREP Plant DNA – 01”.

Figure 7: Version number of the prefilling protocol

19. Check the correct deck position of the plates, reservoirs and other hardware components and confirm with “Ok” (→ see **Figure 8**).

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

20. 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.

21. Remove the CyBio TipRack 96/1000 µL and discard all tips.
22. Remove 8-channel adapter (Head R 96) with Support 37 mm.
23. Discard the reservoirs and their contents.
24. The plates **Plate 3 – Washing Solution A**, **Plate 4 - Washing Solution B2**, **Plate 5 – 80 % Ethanol**, and the **Gripper with Support 37 mm** do not have to be removed for the extraction process.
25. **Plate 7 – RNase free Water (for Elution)** has to be removed from position 1 and placed on position 6.
26. Proceed with ”Preparing the Process Plate” on page 21.

10.2 Manual Prefilling

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

Plate	Label	Content per well
Plate 1	Process	**100 µL** Binding Solution SBS
Plate 2*	Waste	empty
Plate 3	Washing Solution A	**300 µL** Washing Solution A
Plate 4	Washing Solution B2	**300 µL** Washing Solution B2
Plate 5	80% Ethanol	**300 µL** 80% Ethanol
Plate 6	RNase-free Water (for Elution)	**300 µL** RNase-free Water (for Elution)
Plate 7*	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 for the extraction of DNA from plant samples

11.1 Preparing the Process Plate

1. Transfer **4 µL** of **MAG Suspension F** directly into the liquid of each cavity of the prefilled plate **"Plate 1 – Process"**.

NOTE
It is important to mix the **MAG Suspension F** by vigorous shaking or vortexing before use (approx. 30 sec). Repeated vortexing after pipetting 10 samples is recommended.

2. Add 100 µL sample material after centrifugation (→ see "Product Specifications", p.9) into the chosen cavity of the prefilled plate **"Plate 1 – Process"**.

11.2 Loading of 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 6 - 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. 12).

NOTE

Please pay special attention to the following deck positions:
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** and put it on the **97 mm support**. Make sure that every Filter Tip fits into a cavity of the Protective Plate.

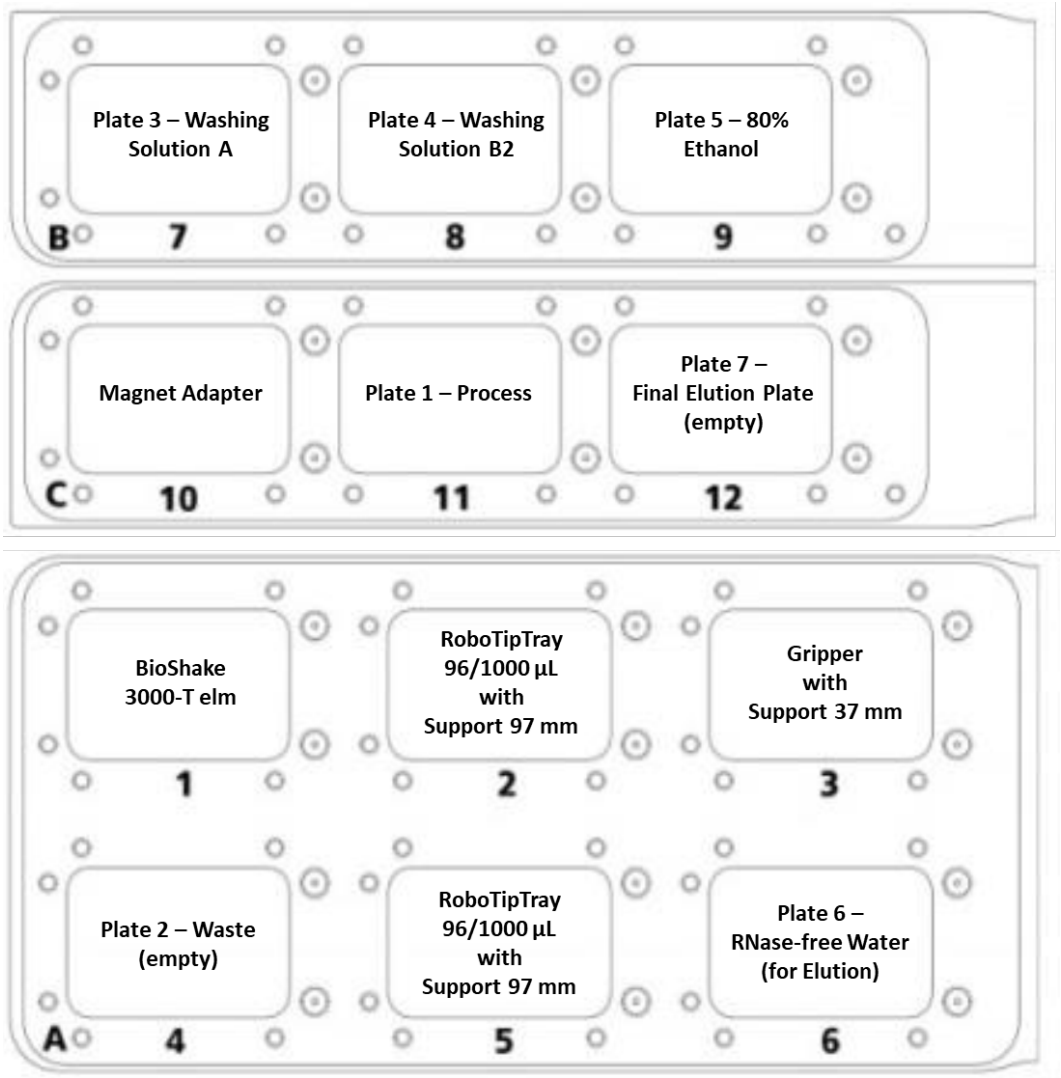

Figure 10: Deck layout for extraction.

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

3. Select “Magnetic Beads” (→ see **Figure 11**).

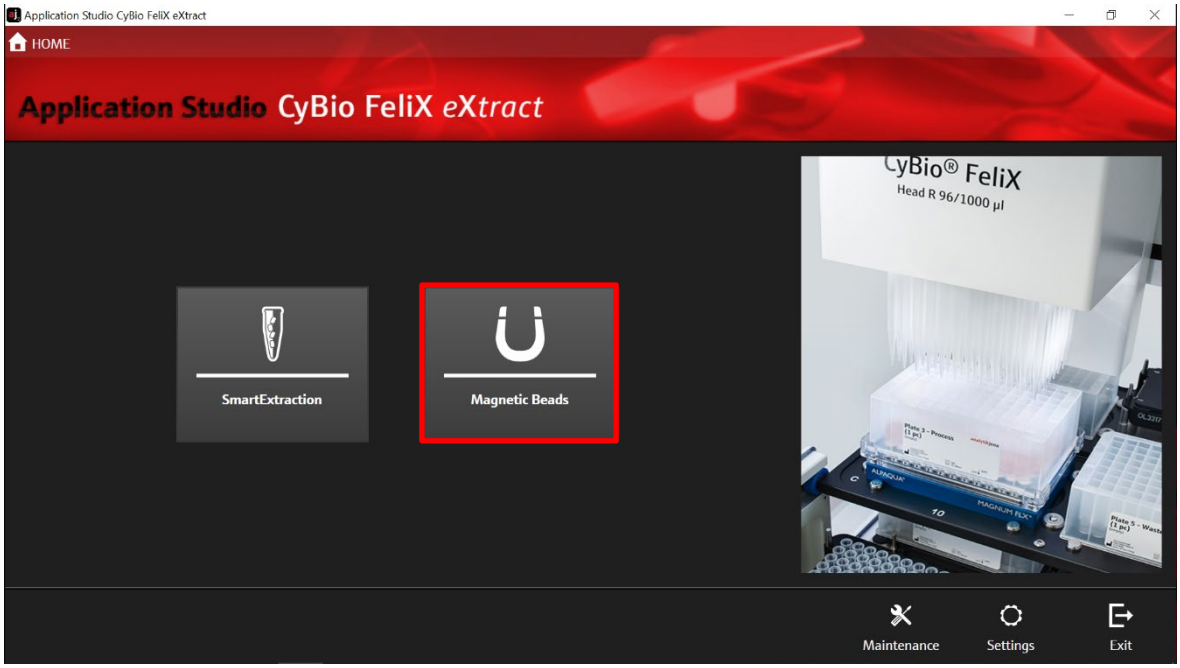

Figure 11: Homescreen of the AppStudio FeliX eXtract. Selection of Magnetic Beads

4. Select “innuPREP Plant DNA Kit – FX” (→ see **Figure 12**).

Figure 12: Kit selection: innuPREP Plant DNA Kit – FX.

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

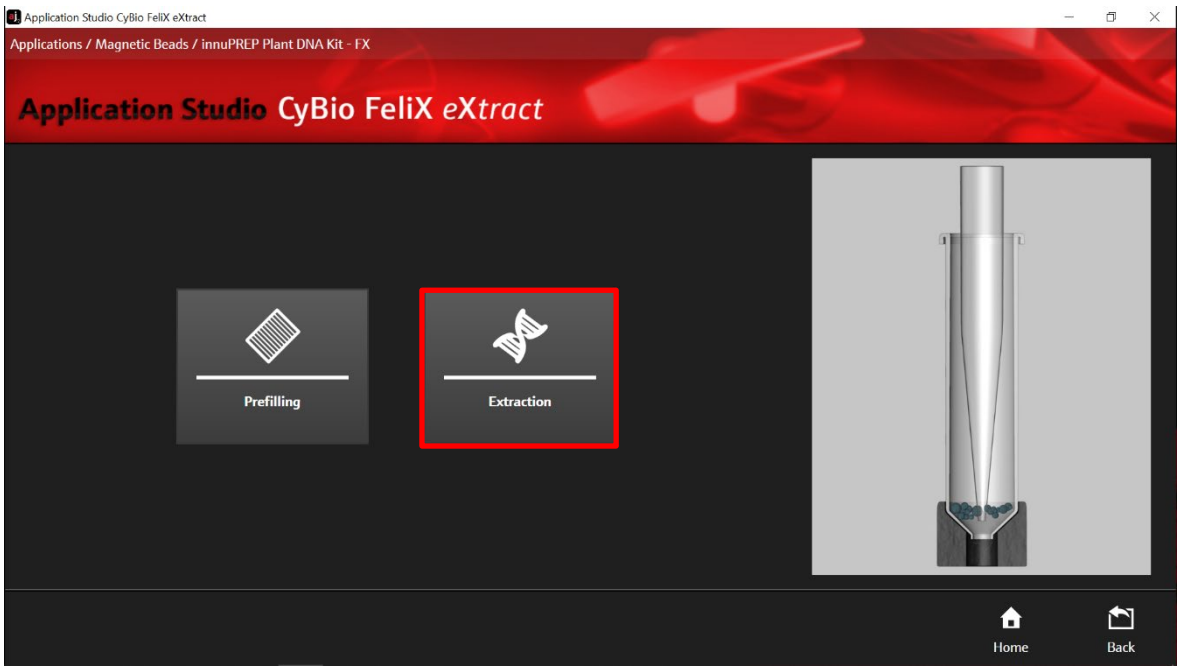

Figure 13: Routine selection: Extraction

6. After selecting “Extraction” the Extraction Start Screen appears (→see **Figure 15**).
7. Check the correct version number of the protocol (→ see **Figure 14**): “Extraction - innuPREP Plant DNA – 01”.

Figure 14: Version number of the extraction protocol.

8. Adjust elution volume (between 50–200 µL, 100 µL are recommended; → see **Figure 15**).

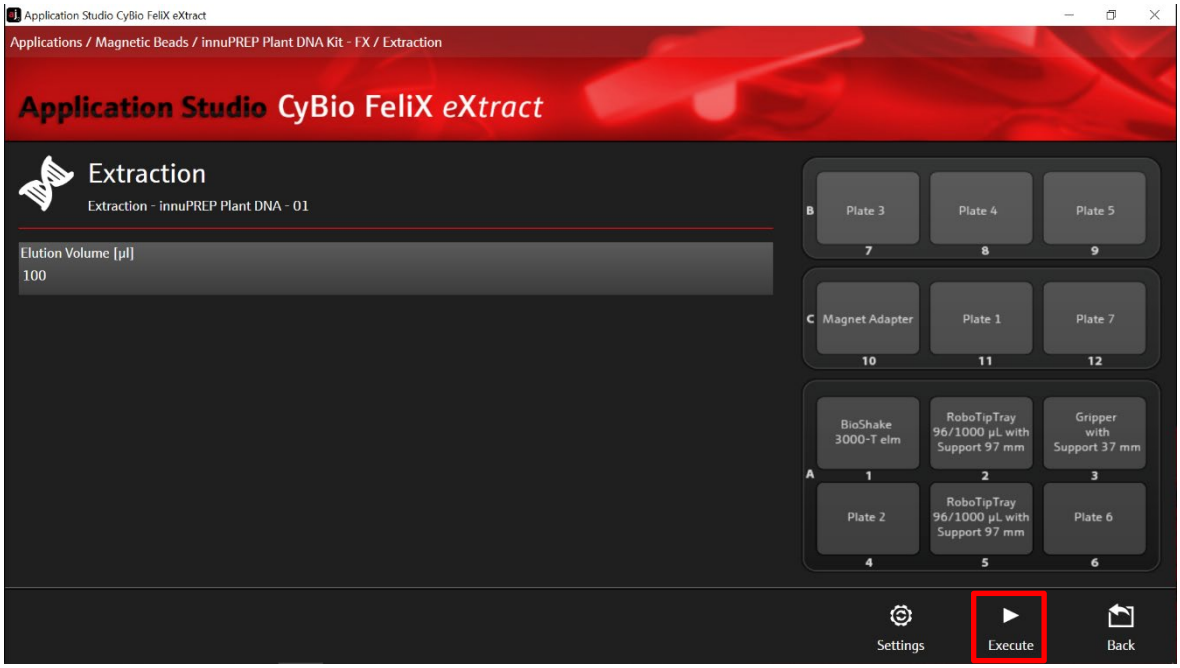

Figure 15: Adjustment of elution volume (variable).

9. After selecting “Execute” the deck layout is shown. Check the correct positions of the plates and accessories and confirm the message with “Ok” to start the protocol (→ see **Figure 16**).

Figure 16: Deck layout for the final hardware check for the extraction.

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

Figure 17: Process completed.

11. Confirm the “Process completed” message with “Ok” (→ see **Figure 17**).
12. Remove **Plate 7 – Final Elution Plate** from **deck position 12** seal it with the included sealing film and store the DNA under adequate conditions.

NOTE

When using alternate elution vessels as listed in section 11.2 “Loading of CyBio Felix” (→ see p. 21), proceed analogously.

Store the DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C. For long-term storage placing at -80 °C is recommended!

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

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted genomic DNA	
No extracted DNA	No magnetic beads added to **Plate 1 - Process**. Please add 4 µL **MAG Suspension F** to **Plate 1 – Process** prior the extraction procedure. Ensure **MAG Suspension F** is mixed well before use.
Insufficient lysis of starting material.	Make sure to use the required volume of 20 µL **Proteinase K**.
Elution volume too high.	Decrease the elution volume. The recommended elution volume is 100 µL. Please note that reducing the 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” on page 9. Use Internal Controls for the verification of 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