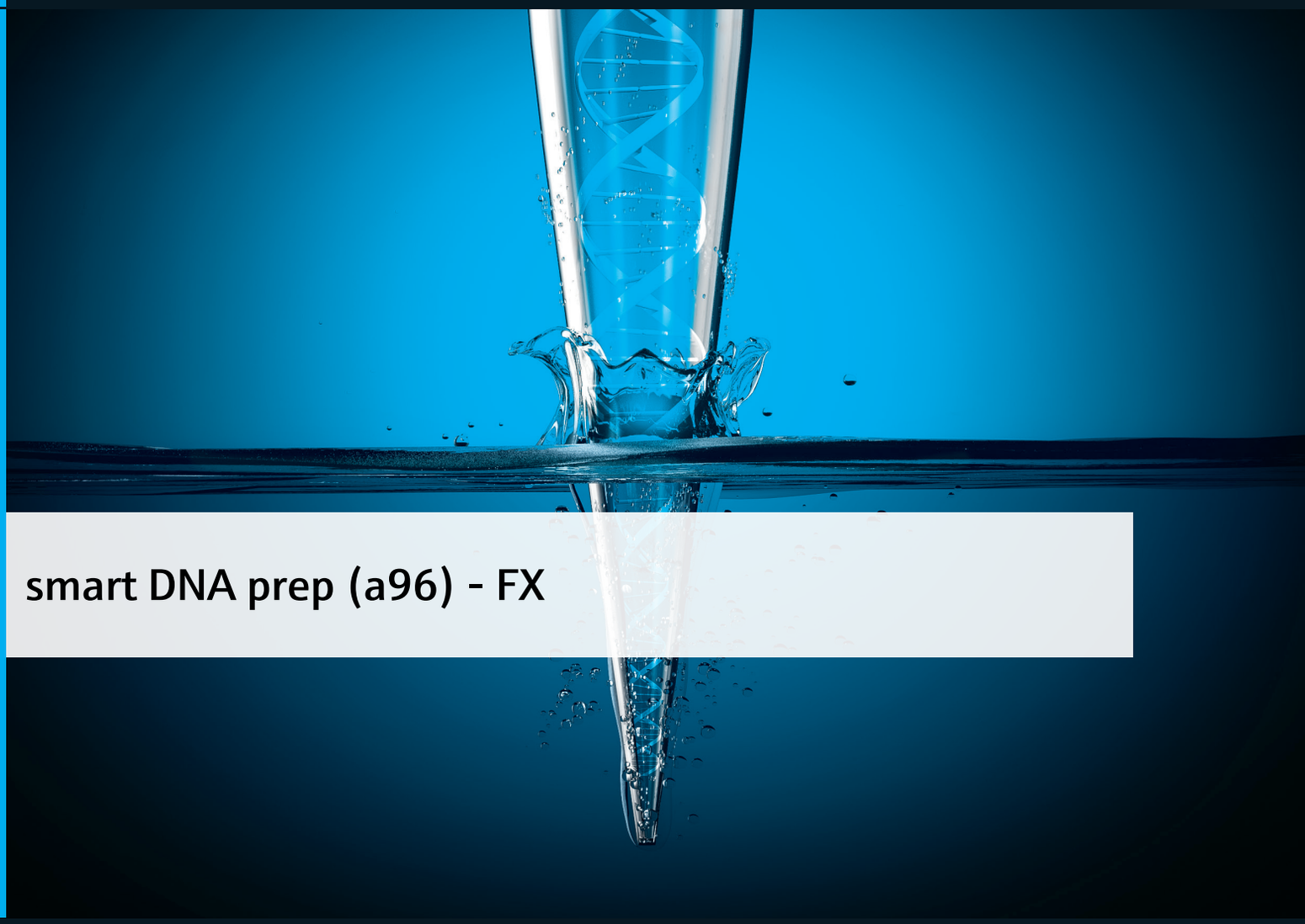
Instructions for Use
Life Science Kits & Assays



smart DNA prep (a96) - FX

Innuscreen GmbH

Order No.:
845-FX-4096096 96 reactions
845-FX-4096480 480 reactions

Publication No.: HB-FX-4096_e_220305

This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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

Contents

1 Introduction

1.1 Intended use

The **smart DNA prep (a96) – FX** has been designed for automated isolation of high molecular weight genomic DNA (gDNA) from tissue samples, eukaryotic cells, rodent tails, bacteria and yeast cells using CyBio FeliX. The kit utilizes the SmartExtraction technology invented by IST Innuscreen GmbH.

The procedure starts with the lysis of the starting material. Following lysis, samples are transferred into the Deep Well Plates of the kit, which have to be prefilled with extraction chemistry.

The extraction process is based on adsorption of the genomic DNA to Smart Modified Surfaces inside the tip. After washing, the genomic DNA is eluted from the Smart Modified Surfaces and is ready for use in subsequent downstream applications.

The whole extraction process simply requires pipetting up and down. The combination of the low-salt DC-Technology with Smart Modified Surface is optimized to get a maximum of yield and quality.

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 the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> tests.
30 °C 15 °C	**Storage conditions** Store at room temperature or shown conditions respectively.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the 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 these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
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 Storage conditions

All components of the kit are shipped at ambient temperature.

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

All other components of the smart DNA prep (a96) - 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, dissolve these by careful warming.

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 product 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 smart DNA prep (a96) – 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.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications” p. 10). 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 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
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 96	Σ 480
REF	845-FX-4096096	845-FX-4096480
SmartExtraction Tips	6 x 16	30 x 16
Proteinase K	for 4 x 1.5 mL working solution	for 18 x 1.5 mL working solution
Lysis Solution CBO	50 mL	2 x 100 mL
Washing Solution LS (conc.)	15 mL	5 x 15 mL
Elution Buffer	70 mL	5 x 70 mL
Deep Well Plate	7	35
Final Elution Plate	1	5
Sealing Foil	1	5
Filter Tips	96	5 x 96
Protective Plate	2	10
Manual	1	1

6.2 Components not included in the kit

- 1.5 mL and 15 mL tubes
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- 80 % Ethanol (molecular biology grade, undenatured)
- 2-Propanol (molecular biology grade)
- 96–99.8 % Ethanol (molecular biology grade, undenatured)
- ddH_2O for dissolving **Proteinase K**
- RNase A (10 mg/mL) for RNA removal (if required)
- 1 column and 3 column reservoirs for prefilling by CyBio FeliX (Smart Prefilling Set 1, 5x96 reactions, OL3317-25-128, Analytik Jena GmbH)

6.2.1 Components needed for isolation from bacteria

- Lysozyme (stock solution 10 mg/mL (400 U/µL))
- Mutanolysin (stock solution 0.4 U/µL)
- Lysostaphin (stock solution 0.4 U/µL)
- TE-Buffer

Alternatively:

- innuPREP Bacteria Lysis Booster (845-KA-1000050, 50 rxn, IST Innuscreen GmbH)

6.2.2 Components needed for isolation from yeasts

- Yeast Digest Buffer (50 mM KH_2PO_4, 10 mM DTT, pH 7.5)
- Lyticase (stock solution 10 U/µL)

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 AppStudio 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-8600115, 115 pcs, IST Innuscreen GmbH)

- Final Elution Plate (96 well, 1.2 mL) (31-01642, 5 pcs, IST Innuscreen GmbH)
- Smart Prefilling Set 1 (OL3317-25-128, 5x96 reactions, Analytik Jena 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

- Tissue samples (1 mg – 100 mg)

  For tissue samples with a high DNA content (e.g. spleen samples, pancreatic samples, lymph nodes) use max. 5 mg!
- Rodent tails (mouse tails up to 1.0 cm, rat tails up to 0.5 cm)
- Eukaryotic cells (max. 5 x 10^6)
- Bacteria cell pellets (1 x 10^5 – 1 x 10^9 cells)
- Yeast cell pellets (1 x 10^5 – 1 x 10^9 cells)

2. Time for isolation

Lysis (external)	Automated prefilling	Extraction	Elution volume
Depends on starting material	21 min	45 min	150–500 µL

3. Typical yield

- Typical yields from murine tissues:

Type	Amount	Typical yield
Liver	5 mg	20 – 40 µg
	20 mg	80 – 150 µg
Lung	5 mg	15 – 25 µg
	20 mg	70 – 100 µg
Kidney	5 mg	15 – 25 µg
	20 mg	60 – 100 µg
Spleen	5 mg	50 – 100 µg
Tail	0.8 cm	40 – 80 µg
NIH 3T3 cells	1 x 10^6 cells	15 – 30 µg

NOTE
Yield of isolated DNA is affected by amount and condition of starting material used. The condition of starting material depends on storage conditions.

8 Initial steps before starting

- Add 1.5 mL of ddH_2O to each vial of **Proteinase K**. Mix thoroughly and store as described above.
- Add 60 mL absolute ethanol to each bottle **Washing Solution LS (conc.)** and mix thoroughly. Keep the bottles always firmly closed.

- 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! Use 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).

9 Prefilling of Reagent Plates

There is the option to prefill the plates automatically (→ see section 9.1) or manually (→ see section 9.2) with the CyBio FeliX.

9.1 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**).

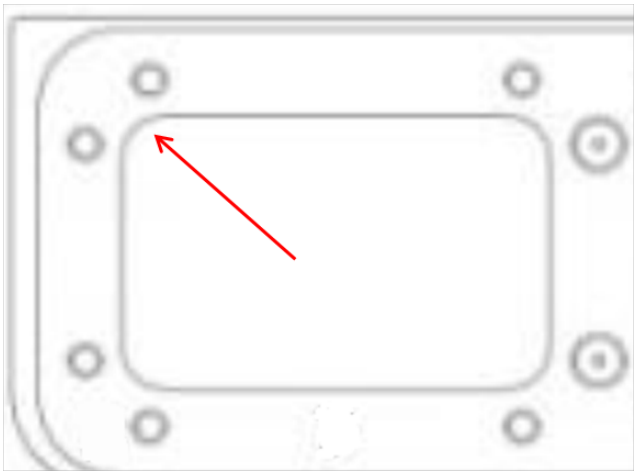


Figure 2: Positioning of plates and reservoirs on CyBio FeliX deck.

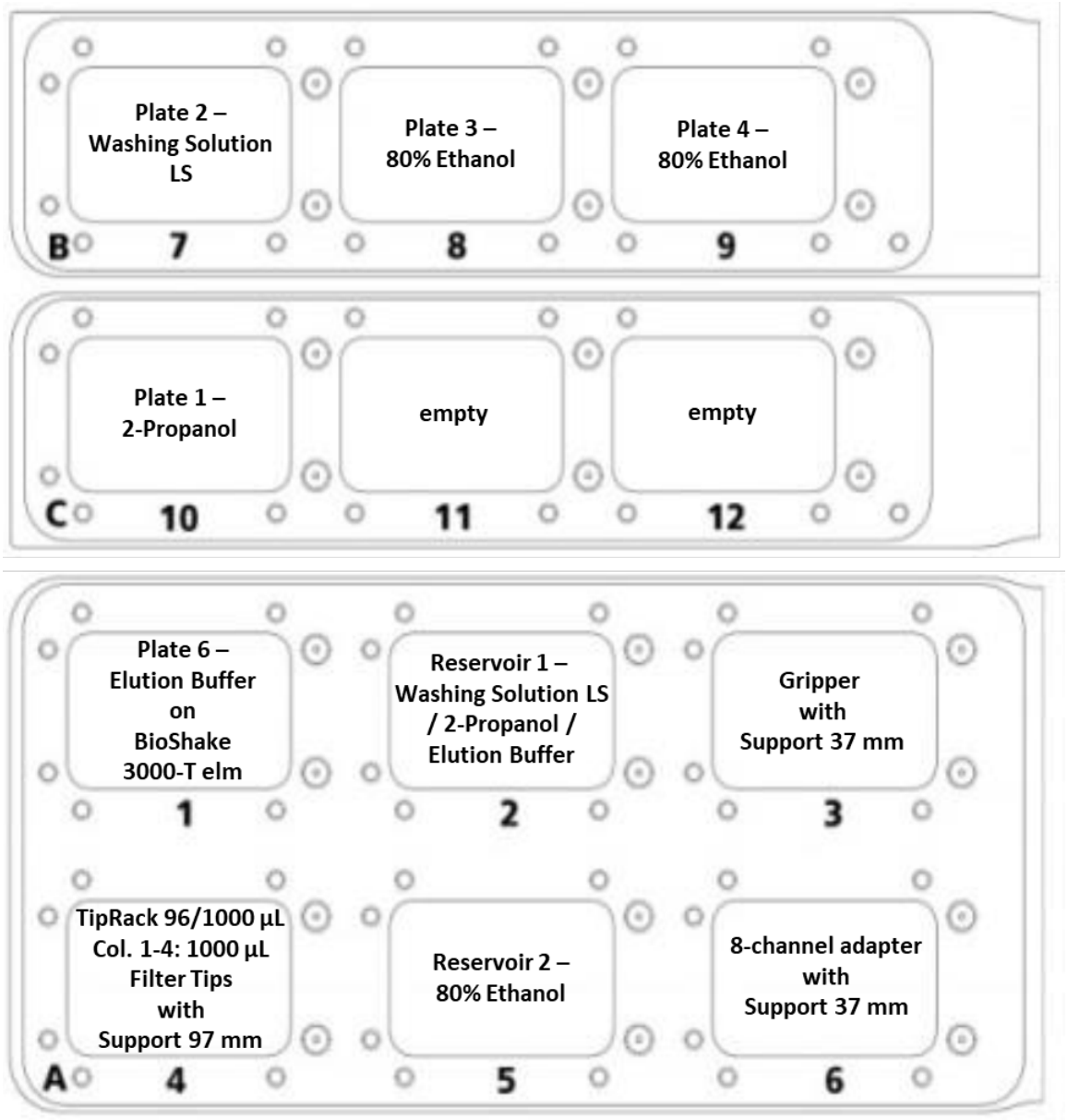


Figure 3: Deck Layout for the prefilling protocol.

NOTE

It is recommended to do the prefilling immediately before the extraction process.

1. Label the 3 column reservoir and the 1 column reservoir from the Smart Prefilling Set 1 according the table below:

Number	Label
Reservoir 1 (3 column)	**Reservoir 1:** Left side of reservoir: Washing Solution LS Middle position: 2-Propanol Right side of reservoir: Elution Buffer
Reservoir 2 (1 column)	**Reservoir 2:** 80 % Ethanol

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

Plate	Label
Plate 1	2-Propanol
Plate 2	Washing Solution LS
Plate 3	80% Ethanol
Plate 4	80% Ethanol
Plate 5*	Drying (empty)
Plate 6	Elution Buffer
Plate 7*	Elution Plate (empty)
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 (75 mL) **Washing Solution LS** into the **left** side of the 3 column reservoir labeled “Reservoir 1 –Washing Solution LS/ 2-Propanol/ Elution Buffer”.
4. Transfer 40 mL **2-Propanol** into the **middle position** of the 3 column reservoir labeled “Reservoir 1 –Washing Solution LS/ 2-Propanol/ Elution Buffer”.
5. Transfer the content of the bottle (70 mL) **Elution Buffer** into the **right** side of the 3 column reservoir labeled “Reservoir 1 –Washing

Solution LS/ 2-Propanol/ Elution Buffer". Place the filled reservoir into the CyBio FeliX on position 2 (→ see **Figure 3**).

6. Transfer 130 mL **80% Ethanol** into the 1 column reservoir labeled "Reservoir 2 – 80 % Ethanol". Place the filled reservoir into the CyBio FeliX on position 5 (→ see **Figure 3**).
7. Insert filter tips in columns 1-4 in the CyBio Tip Rack 96/1000 µL. Please fill the whole rows of the columns with Filter Tips.
8. Place the CyBio Tip Rack 96/1000 µL into the CyBio FeliX on position 4 (→ see **Figure 3**).
9. Place the 8-channel adapter (Head R 96) with the support 37 mm into the CyBio FeliX on position 6 (→ see **Figure 3**).
10. Place the empty, labeled plates on the CyBio FeliX deck according to deck layout 1 (→ see **Figure 3**).

NOTE
Please pay special attention to the following deck position:
Position 1:
Place **Plate 6 – Elution Buffer** directly on the BioShake 3000-T elm.

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

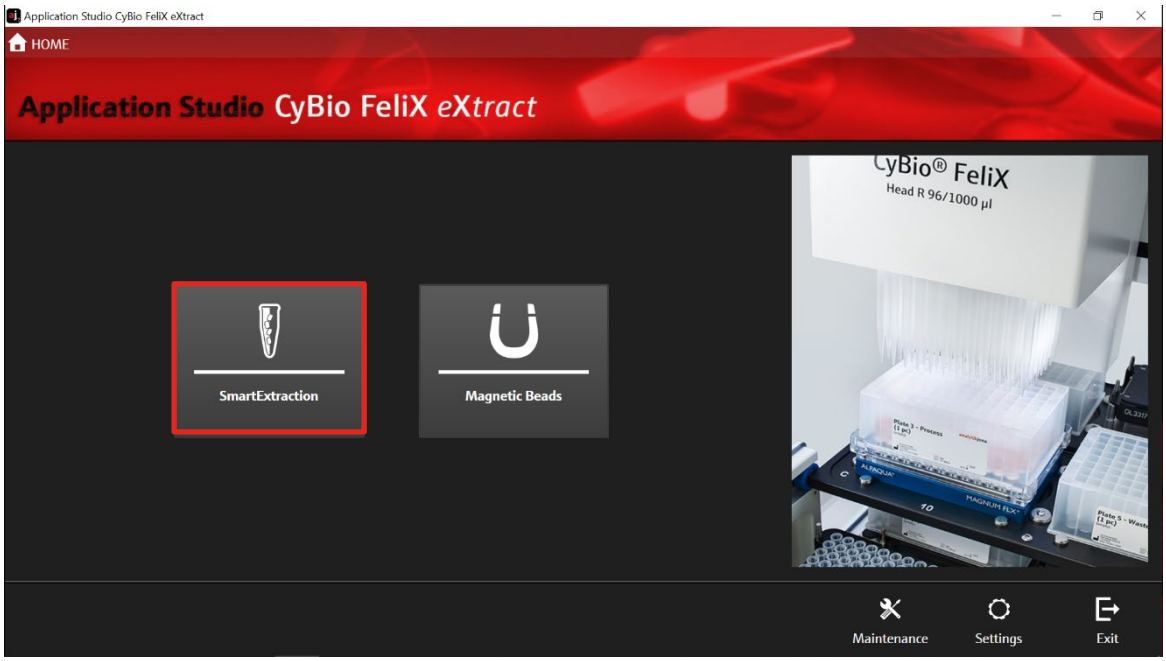


Figure 4: HomeScreen of the AppStudio FeliX eXtract. Selection of extraction technology: "SmartExtraction".

13. Choose “smart DNA prep (a96) – FX” (→ see **Figure 5**).

Figure 5: Kit selection: smart DNA prep (a96).

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

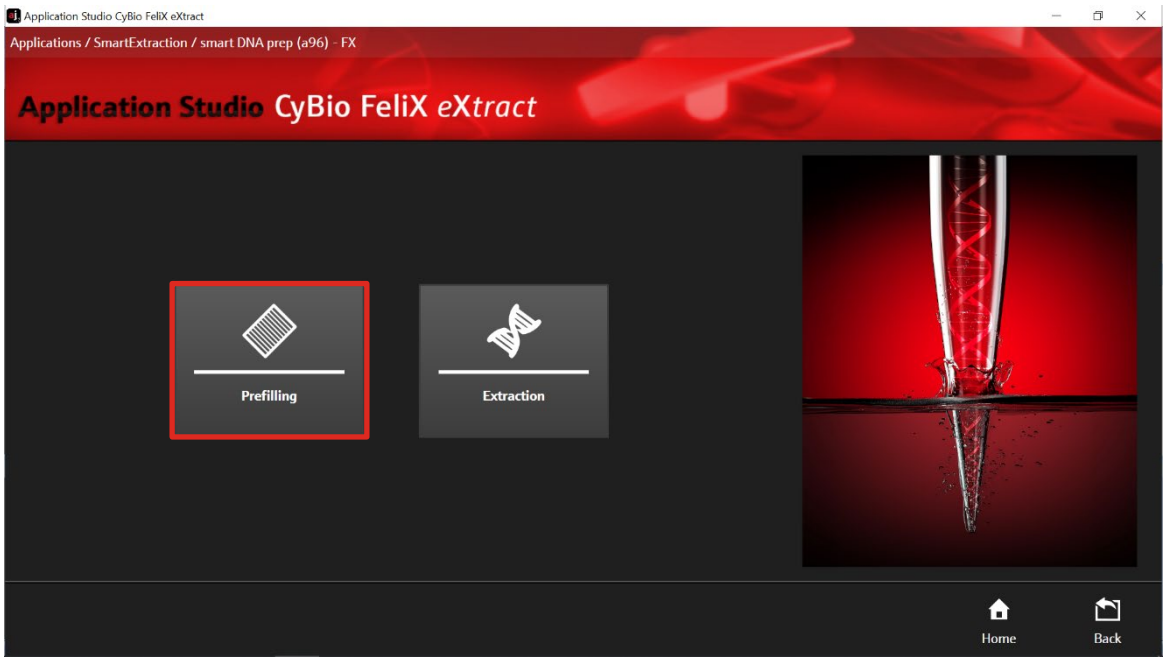


Figure 6: Routine selection: Prefilling.

15. After choosing “Prefilling” the Prefilling Start Screen appears.
16. Check the correct version number of the protocol (→ see **Figure 7**): “Prefilling – SE External Lysis (a96) – 01”.

Figure 7: Version number of the prefilling protocol.

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

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

18. The chosen protocol is performed by the device. After the protocol is finished, the message “Prefilling completed” is shown. Confirm the message with “Ok” (→ see **Figure 9**).

Figure 9: The prefilling process is completed.

19. Remove the CyBio TipRack 96/1000 µL and discard all tips.
20. Remove 8-channel adapter (Head R 96) with Support 37 mm.
21. Discard the reservoirs and all their contents.
22. The plates **Plate 2- Washing Solution LS**, **Plate 3 – 80% Ethanol**, **Plate 4 – 80% Ethanol** and the **Gripper with Support 37 mm** do not have to be removed for the extraction process, all other plates have to be removed.

9.2 Manual prefilling of Reagent Plates

NOTE
Prefilling of Deep Well Plates can be carried out during lysis of samples. Ensure that Washing Solution LS has been prepared according to the instructions (→ see "Initial steps before starting", p. 11).
80 % Ethanol and 2-Propanol are not supplied with the kit.

Label and fill the **Deep Well Plates** according to the table below.

Plate	Label	Content
Plate 1	2-Propanol	**350 µL** 2-Propanol
Plate 2	Washing Solution LS	**600 µL** Washing Solution LS
Plate 3	80 % Ethanol	**600 µL** 80 % Ethanol
Plate 4	80 % Ethanol	**600 µL** 80 % Ethanol
Plate 5*	Drying Plate	**empty**
Plate 6	Elution Buffer	**600 µL** Elution Buffer
Plate 7*	Elution Plate	**empty**
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 have to be prefilled.

NOTE
The lysed sample will be processed using CyBio FeliX as a liquid handling platform. Please pay special attention to section 11.2 "Loading the sample and starting CyBio FeliX" on p.26.

10 Sample preparation

NOTE
Ensure that **Proteinase K** has been prepared according to the instructions on page 10 (→ see "Initial steps before starting" on p. 11).

10.1 Sample preparation of tissue samples and rodent tails

1. Cut the tissue sample into small pieces and place them in a 1.5 mL reaction tube.
2. Add **400 µL Lysis Solution CBO** and **40 µL Proteinase K**, mix vigorously by pulsed vortexing for 5 sec.
3. Incubate at 55 °C in a shaking platform until the sample is lysed. Sample lysis time depends on amount and kind of sample and should be completed within 0.5 – 3 h.

NOTE
We recommend using a shaking platform for continuous shaking of the sample. Alternatively, vortex the sample every 10 min during the incubation. No shaking will reduce the lysis efficiency.

OPTIONAL
To remove RNA from the sample (if required) add 1 µL of RNase A solution (10 mg/mL) after the lysis step, vortex shortly and incubate for 10 min at room temperature. Be sure that RNase A is free of DNase-activity.

4. After lysis centrifuge the 1.5 mL tube at 10,000 x g (~ 12,000 rpm) for 2 min to spin down unlysed material.
5. Use **400 µL** of the supernatant for the automated extraction (→ see section 11.2 on p. 26).

IMPORTANT
Depending on the sample used, a thin solid phase on the surface of the centrifuged sample can be observed. Avoid the contamination of the sample with this solid phase (e.g. fat).

10.2 Sample preparation of eukaryotic cells

1. Pellet eukaryotic cells by centrifugation for 10 min at 5,000 x g (~7,500 rpm). Discard the supernatant.
2. Add **200 µL** 1 x PBS Buffer to the cell pellet and resuspend the cell pellet completely by pipetting up and down.
3. Add **200 µL Lysis Solution CBO** and the required amount of **Proteinase K** (see table below), mix vigorously by pulsed vortexing for 5 sec.

Number of cells	Proteinase K to be added
Up to 1 x 10^6	20 µL
1–5 x 10^6	40 µL

4. Incubate at 55 °C for 30 min.

NOTE
We recommend using a shaking platform for continuous shaking of the sample. Alternatively, vortex the sample every 10 min during the incubation. No shaking will reduce the lysis efficiency.

OPTIONAL
To remove RNA from the sample and add 1 µL of RNase A solution (10 mg/mL) after the lysis step, vortex shortly and incubate for 5 minutes at room temperature. Be sure that RNase A is free of DNase-activity.

5. Use **400 µL** of the supernatant for the automated extraction (→ continue according to section 11 ”Automated DNA extraction using CyBio FeliX” on p.26).

IMPORTANT
Depending on the sample used, a thin solid phase on the surface of the centrifuged sample can be observed. Avoid the contamination of the sample with this solid phase (e.g. fat).

10.3 Sample preparation of bacteria cell pellets

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 min at 3,000 x g) and discard the supernatant.
2. Resuspend the bacterial cell pellet in **170 µL TE Buffer**. After resuspension start enzymatic pre-lysis as described below. Requirements for pre-lysis depend on the cell type.

10.3.1 Pre-lysis of resuspended starting material

10.3.1.1 Gram-negative bacteria

Although gram-negative bacteria do not require a pre-lysis step, using Lysozyme (not included in the kit) can enhance the efficiency of lysis.

Using Lysozyme: stock solution of Lysozyme: 10 mg/mL (400 U/µL)

3. Add **20 µL** Lysozyme to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.

   Proceed with “Proteolytic lysis step” on p. 25.

10.3.1.2 Staphylococcus spp.

For lysis of *Staphylococcus* the enzyme Lysostaphin is recommended (not included in the kit).

Using Lysostaphin: stock solution of Lysostaphin: 0.4 U/µL

3. Add **10 µL Lysostaphin** to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.
4. Proceed with “Proteolytic lysis step” on p. 25.

Alternatively: use the **innuPREP Bacteria Lysis Booster**

The innuPREP Bacteria Lysis Booster Kit has been developed for a highly efficient pre-lysis of bacterial cell walls thus generating spheroplasts. This new mixture of different enzymes boosts the lysis of all bacteria in particular hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium*.

1. Prepare the enzyme mix according to the manual of the **innuPREP Bacteria Lysis Booster**.

2. Add **20 µL of the prepared enzyme** mix to the sample and vortex it shortly. Incubate the sample for 30 min at 37 °C.

Proceed with “Proteolytic lysis step” on p. 25.

10.3.1.3 Gram-positive bacteria

Gram-positive bacteria require a pre-lysis step using Mutanolysin and/or Lysozyme (not included in the kit).

Using Lysozyme: stock solution of Lysozyme: 10 mg/mL (400 U/µL)

3. Add **20 µL Lysozyme** to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.

Using Mutanolysin: stock solution of Mutanolysin: 0.4 U/µL

4. Add **5 µL** Mutanolysin to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.
5. Proceed with “Proteolytic lysis step” on p. 25.

NOTE
Lysozyme and Mutanolysin exert synergistic activity. Using both enzymes together will increase the yield of isolated nucleic acids.

Alternatively: use **innuPREP Bacteria Lysis Booster**.

Proceed as described in section 10.3.1.2 Staphylococcus on page 23.

10.4 Sample preparation of yeast cells

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 min with 3,000 x g) and discard the supernatant.
2. Resuspend the yeast cell pellet in **200 µL Yeast Digest Buffer** (→ see ”Components needed for isolation from yeasts”, p. 9).
3. **Enzymatic pre-lysis** (enzyme is not included in the kit) is recommended

Using Lyticase: stock solution of Lyticase is 10 U/µL

4. Add **10 µL Lyticase** to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.

5. Proceed with “Proteolytic lysis step” on p. 25.

10.5 Proteolytic lysis step

1. Add **200 µL Lysis Solution CBO** and **30 µL Proteinase K** to the sample and mix vigorously by pulsed vortexing for 5 sec.
2. Incubate sample for 30 min at 55 °C and 550 rpm in a shaking platform. Lysis time of 30 min is often sufficient to get enough DNA. If the sample is not clear after 30 min, prolong the incubation time until the sample is clear.

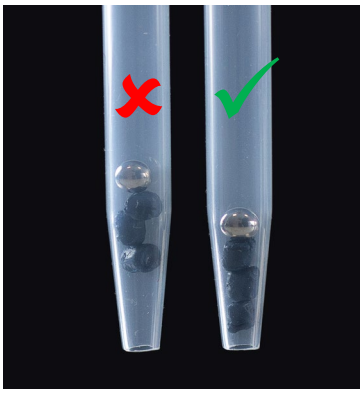
NOTE
To remove RNA from the sample (if required) add 1 µL of RNase A solution (10 mg/mL), vortex shortly and incubate for 10 min at room temperature. Be sure that the RNase A is free of DNase-activity.

3. Use **400 µL** of the supernatant for the automated extraction
(→ see section 11.2 on p. 26)

11 Automated DNA extraction using CyBio FeliX

11.1 Handling of SmartExtraction Pipette Tips

Add 96 SmartExtraction Tips (or the number of tips required) to a 96-Channel Magazine placed on a 97 mm support on **deck position 4**.



Checking SmartExtraction Tips.
Make sure that the Smart Modified Material is collected near the outlet of the SmartExtraction Tip. If necessary, invert the tip a few times or flick it with your fingers or against the edge of a table. The optimal position of the Smart Modified Material inside the tip is shown in **Figure 10**.

Figure 10: Checking SmartExtraction Tips.

11.2 Loading the sample and starting CyBio FeliX

NOTE
The following steps will be done after sample lysis!

1. Transfer the lysate (max. **400 µL**) into **Plate 1 – 2-Propanol.**
2. Put **Plate 1 – 2-Propanol** on **deck position 10**.
3. Place all required plates and accessories onto the CyBio FeliX decks according to **Figure 11**. As a final elution plate (**deck position 12**) multiple options are possible:

   - **Plate 8 – Final Elution Plate**
   - Micronic 750 µL pre-capped and racked 2D-tubes (MP52706-Y20)
   - Greiner Cryo.S 600 µL pre-racked (977561, 977580)

NOTE
Please pay special attention to the following deck positions:
Position 1: **Place Plate 5 – Drying Plate (empty)** on the BioShake 3000-T-elm (**deck position 1**).
Position 4 and 6: Put the **Protective Plate** directly on the bottom of the **97 mm support**.

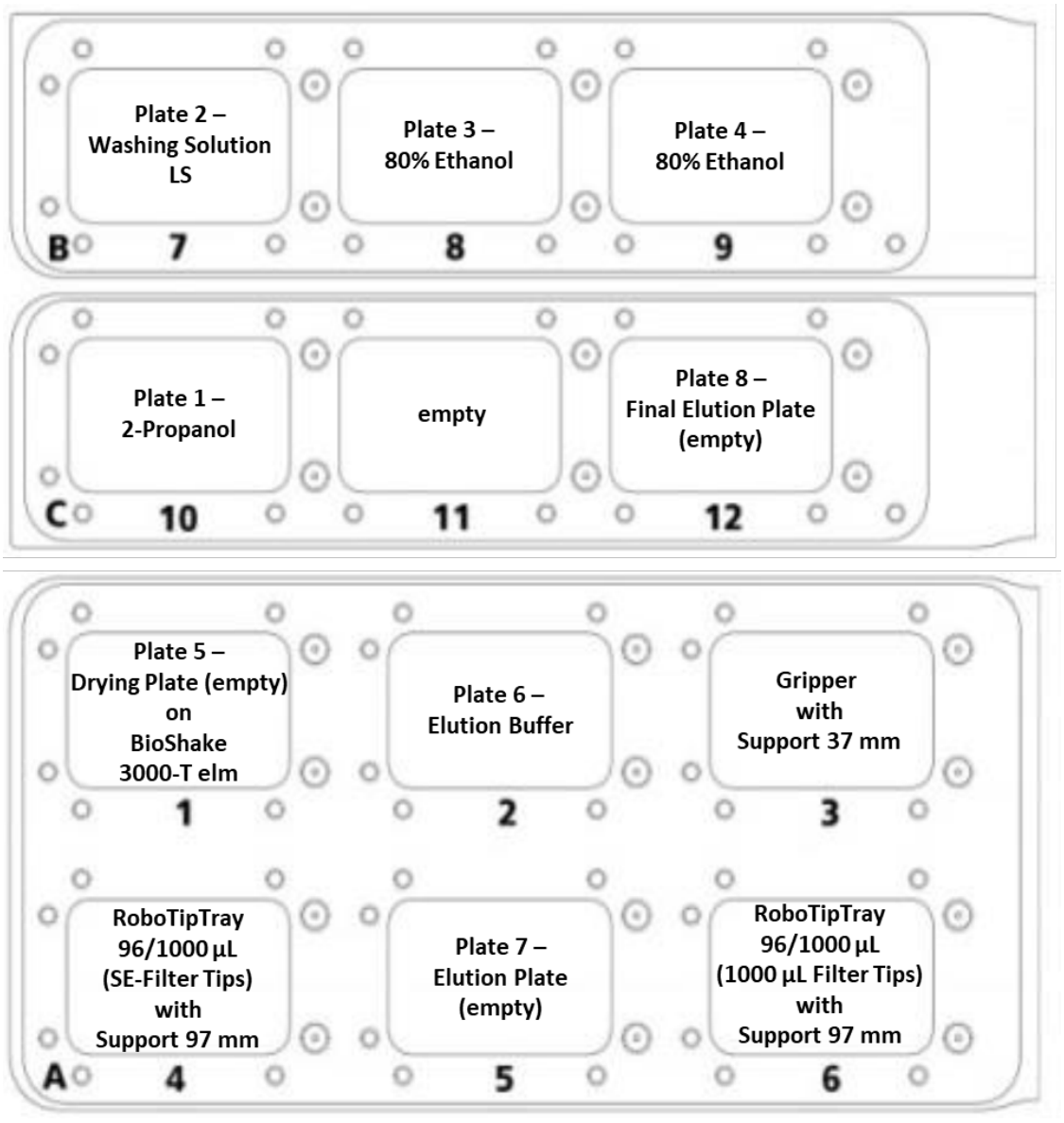


Figure 11: Deck layout for extraction

NOTE

Extracted high molecular weight DNA from large sample amounts tends to be very viscous.

As the extraction protocols include a homogenization step, the fragment size of extracted DNA is reduced. This is suited for downstream applications which do not require high molecular weight DNA.

If downstream application requires high molecular weight DNA, the **CyBio RoboTipTray** must be put at **deck position 6** but has to be left empty and not be equipped with standard filter tips. As a result, the eluate will remain in **Plate 7 – Elution Plate** at the end of the protocol. In this case **Plate 8 – Final Elution Plate** does not have to be placed on **deck position 12**. Transfer of the eluate into storage tubes (e.g. Elution Tubes with Elution Caps, 1.5 mL reaction tubes) must be done manually. In order to avoid loss of DNA integrity pipet carefully with a wide-bore or cut tip.

4. Switch on CyBio FeliX and open the AppStudio FeliX *eXtract*.
5. Select the extraction technology “SmartExtraction” (→ see **Figure 12**).

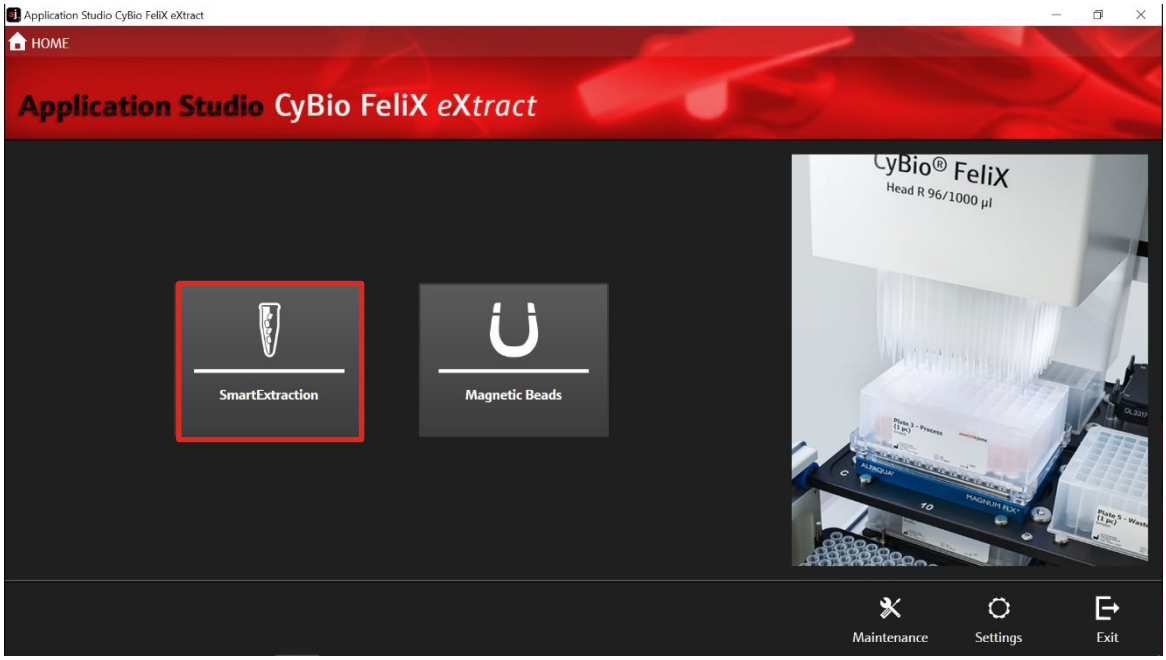


Figure 12: Homescreen of AppStudio FeliX eXtract. Selection of extraction technology: SmartExtraction.

6. Select the kit protocol “smart DNA prep (a96) – FX” (→ see **Figure 13**).

Figure 13: Selection of extraction kit: smart DNA prep (a96) – FX.

7. Select “Extraction” (→ see **Figure 14**).

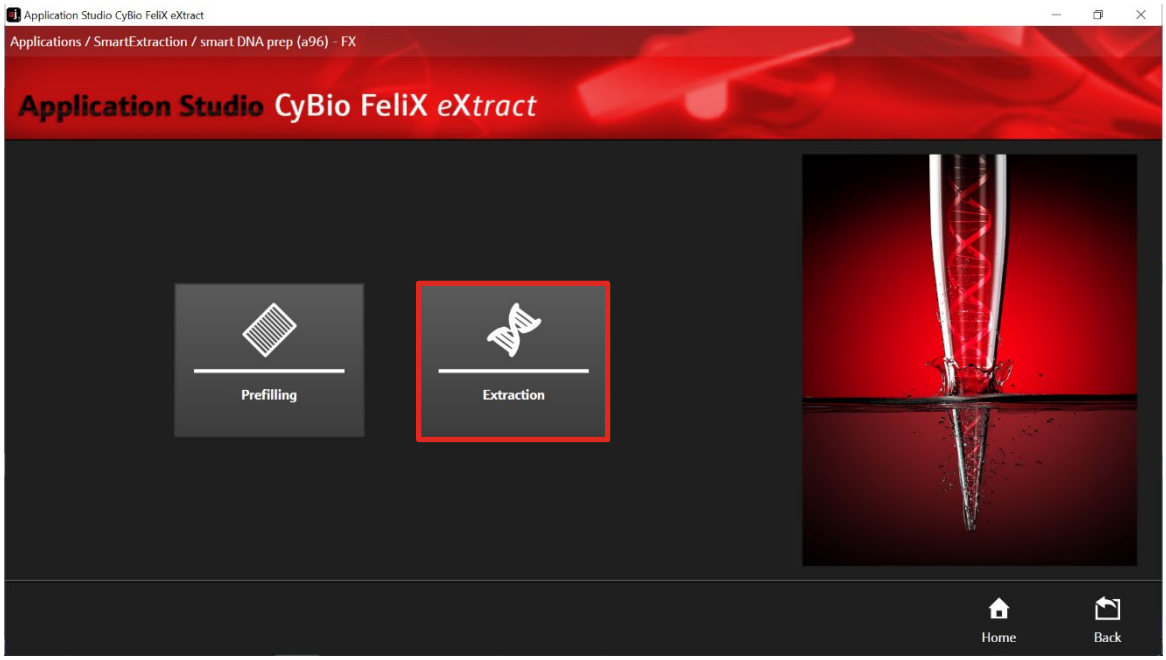


Figure 14: Routine selection: Extraction.

8. After selecting Extraction, the Extraction Start Screen appears (→ see **Figure 16**).

9. Check the right protocol version “External Lysis (a96) – 02” (→ see Figure 15).

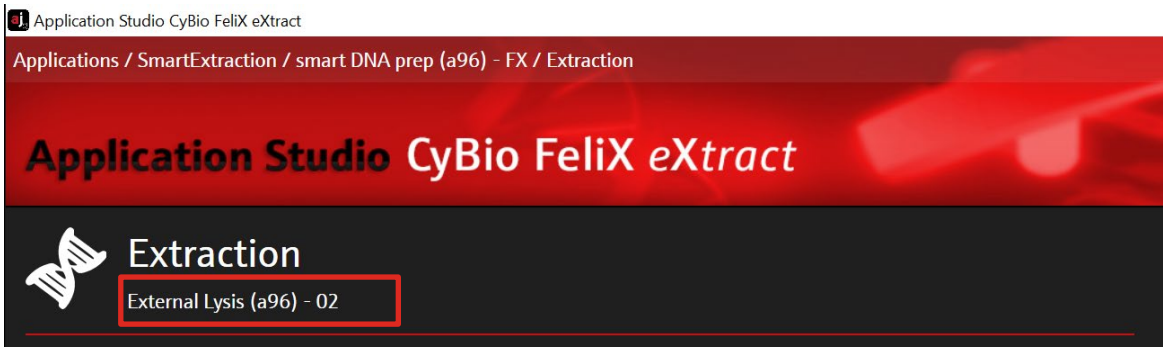


Figure 15: Version number of the extraction protocol.

10. Adjust the “Elution Volume” to 150-500 µL according to the table below. Start the protocol by clicking the button “Execute” (→ see **Figure 16**).

Amount of starting material	Recommended elution volume
< 1 x 10^6 eukaryotic cells	300 µL
1–5 x 10^6 eukaryotic cells	400–500 µL
< 1 x 10^8 bacteria cells	min. 200 µL
> 1 x 10^8 bacteria cells	min. 300 µL
< 5 x 10^8 yeast cells	min. 200 µL
> 5 x 10^8 yeast cells	min. 300 µL

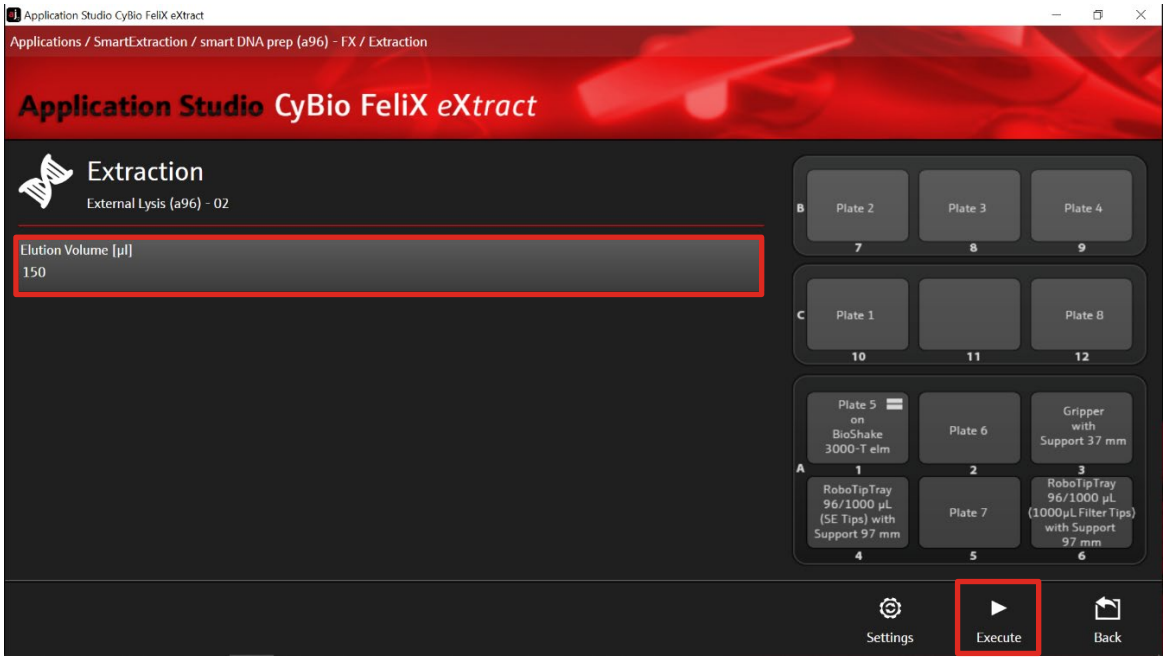


Figure 16: Adjustment of elution volume.

11. After selecting “Execute” the deck layout is shown. Check the correct positioning of plates and accessories on the corresponding deck positions and confirm with “Ok” (→see **Figure 17**).

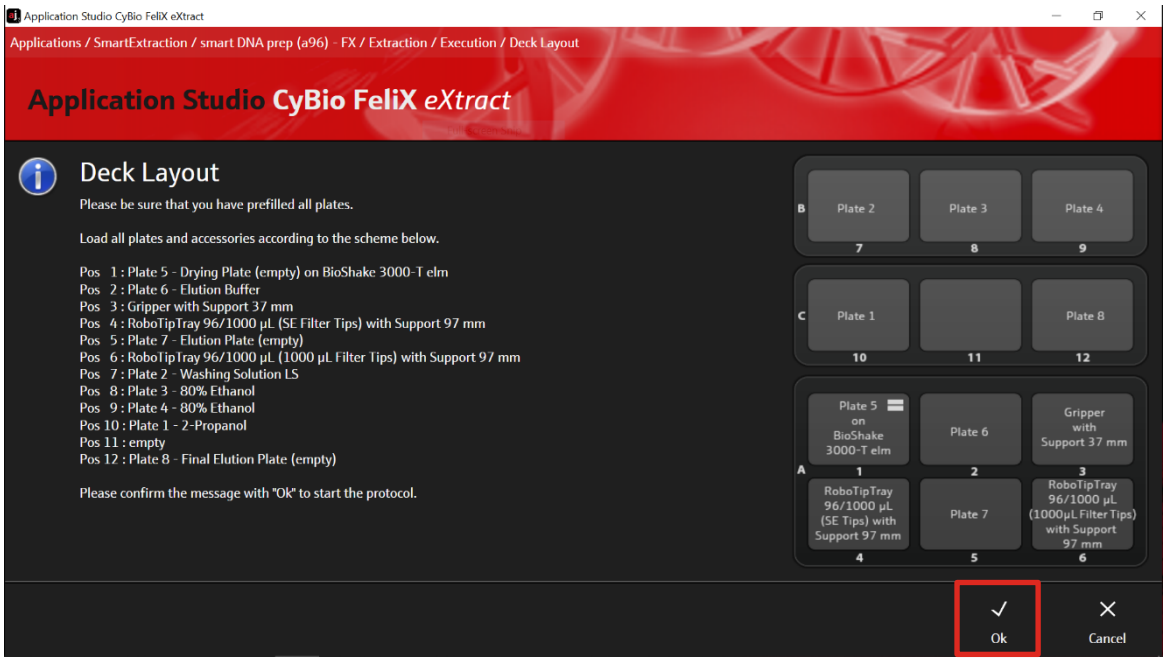


Figure 17: **Deck layout for checking the correct positions of all plates and accessories.**

12. The chosen protocol is performed by device. After the protocol is finished, the message “Purification process completed” is displayed. Confirm the message with “Ok” (→ see Figure 18).

Figure 18: Purification process completed.

13. Once the extraction protocol is finished, remove **Plate 8 – Final Elution Plate** from **deck position 12** or **Plate 7 – Elution Plate** (→ see Note on p. 28) from the BioShake 3000 T-elm at **deck position 1**.

NOTE

When using alternate elution vessels as listed in (→ see "Loading the sample and starting CyBio FeliX", p. 26), proceed analogously.
Store the DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C. For long-term storage we recommend -80 °C.

14. Afterwards, remove and discard the used Deep Well Plates as well as the used tips.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material.
Smart Modified Material not collected near the tip opening	Invert the tip a few times or flick the tip with your fingers or against the edge of a table to collect granulates in the lower part of pipette tip (→ see section 11.1 on p. 26).
High viscosity extracted DNA	
Insufficient amount of Elution Buffer	Elute DNA with a higher volume of Elution Buffer.
Degraded or sheared DNA	
Old material unsuitable	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

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