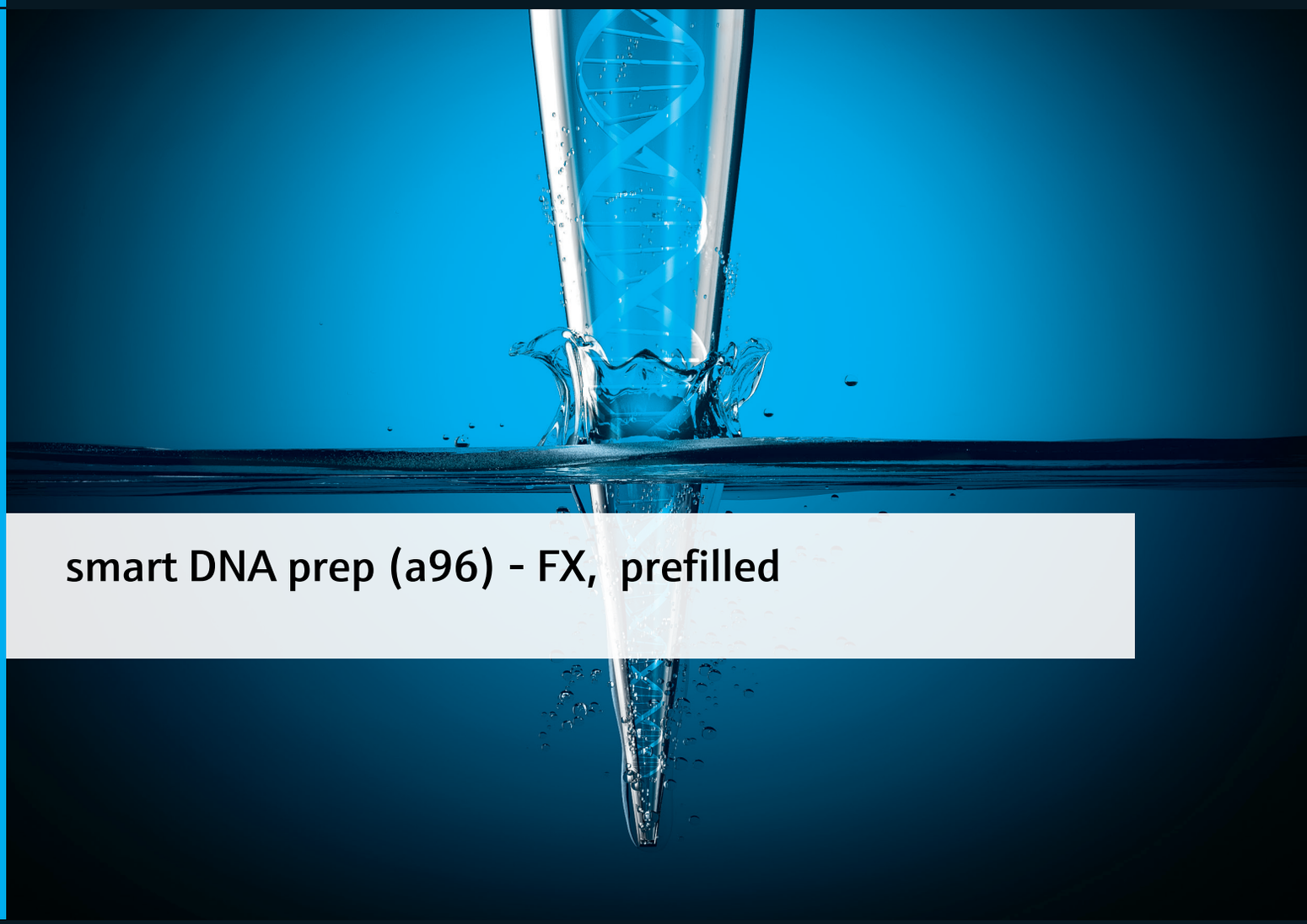
Instructions for Use
Life Science Kits & Assays



smart DNA prep (a96) - FX, prefilled

Innuscreen GmbH

Order No.:
845-PFX-4096096 96 reactions

Publication No.: HB_PFX-4096_e_230815

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 **smart DNA prep (a96) – FX, prefilled kit** 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 new SmartExtraction technology invented by IST Innuscreen GmbH. The plates are already prefilled with the extraction reagents needed for the extraction process.

The procedure starts with the lysis of the starting material. Following lysis, samples are transferred into the Deep Well Plates of the kit, which are already prefilled with extraction chemistry. Therefore, hands-on-time for preparation is reduced to a minimum.

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 patented, low-salt DC-Technology with patent-pending 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 uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number.
	**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 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 prior to 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

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

All other components of the **smart DNA prep (a96) – FX, prefilled** kit 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, prefilled** kit or other IST Innuscreen GmbH products, please do not hesitate to contact us.
For technical support or further information in Germany please contact info.innu@ist.com. For other countries please contact 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 just 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
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 96
REF	845-PFX-4096096
SmartExtraction Tips	6 x 16
Proteinase K	for 4 x 1.5 mL working solution
Lysis Solution CBO	50 mL
Plate 1 – 2-Propanol (350)	1
Plate 2 – Washing Solution LS (600)	1
Plate 3 – 80 % Ethanol (600)	1
Plate 4 – 80 % Ethanol (600)	1
Plate 5 – Drying Plate (empty)	1
Plate 6 – Elution Buffer (600)	1
Plate 7 – Elution Plate (empty)	1
Plate 8 – Final Elution Plate (empty)	1
Sealing Foil	1
Protective Plate	2
Filter Tips	96
Manual	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)
- ddH_2O for dissolving **Proteinase K**
- RNase A (10 mg/mL) for RNA removal (if required)

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)

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 (31-01641, 10 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, 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:

- Tissue sample (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)	Extraction	Elution volume
Depends on starting material	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 ddH_2O to each vial of **Proteinase K**. Mix thoroughly and store as described above.
- 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 Sample preparation

NOTE

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

9.1 Sample preparation and tissue samples and rodent tails

1. Cut the tissue sample into small pieces and place it 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 a continuous shaking of the sample. Alternatively, vortex the sample every 10 min during the incubation. No shaking will reduce the lysis efficiency.

OPTIONAL

If required, RNA can be removed from the sample by adding 1 µL RNase A solution (10 mg/mL) after the lysis step. Vortex the sample 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 (→ continue according to section 10 "Automated DNA extraction using CyBio FeliX" on p.17).

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).

9.2 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 needed 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 a continuous shaking of the sample. Alternatively, vortex the sample every 10 min during the incubation. No shaking will reduce the lysis efficiency.

OPTIONAL
If required, RNA can be removed from the sample by adding 1 µL RNase A solution (10 mg/mL) after the lysis step. Vortex the sample shortly and incubate for 10 min 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 10 ”Automated DNA extraction using CyBio FeliX” on p. 17).

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).

9.3 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 bacteria 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.

9.3.1 Pre-Lysis of resuspended starting material

9.3.1.1 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 is 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. 16.

9.3.1.2 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. 16.

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.
3. Proceed with "Proteolytic lysis step" on p. 16.

9.3.1.3 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 is 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 is 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. 16.

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

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

Proceed as described in section 9.3.1.2 Staphylococcus on page 14.

9.4 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. 16.

9.5 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.

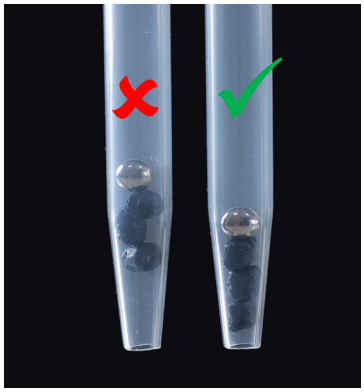
NOTE
If required, RNA can be removed from the sample by adding 1 µL RNase A solution (10 mg/mL) after the lysis step. Vortex the sample shortly and incubate for 10 min at room temperature. Be sure that RNase A is free of DNase-activity.

3. Proceed with “Preparation of Reagent Plates” on p. 17.

10 Automated DNA extraction using CyBio FeliX

10.1 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** (→ see **Figure 3**, p. 19).



Checking the 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 2**.

Figure 2: Checking SmartExtraction Tips.

10.2 Preparation of Reagent Plates

10.2.1 Unpacking of Reagent Plates

NOTE
According to transport regulations Reagent Plates are sealed in plastic bags only when transported by airplane.

Reagent Plates may be delivered wrapped in plastic bags for transport protection.

Carefully open the packaging of Reagent Plates using scissors.

10.2.2 Removing of sealing foil

Reagent Plates are prefilled with extraction reagents and are sealed with a foil. Prior to use, this foil has to be peeled of manually.

Invert each Reagent Plate 3 to 4 times and thump it onto a table to collect the pre-filled solutions at the bottom of the wells. Keep the reagent plates in a horizontal position to avoid spilling of the reagents while peeling the foil. Always wear gloves!

NOTE
Please note that every required cavity must be opened otherwise the protocol run may be aborted.

10.3 Loading the sample and starting CyBio FeliX

NOTE
The following steps will be done after the 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 3**. 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 BioShake 3000-T-elm (**deck position 1**).
Position 4 and 6: Put the **Protective Plate** directly on the bottom of the **97 mm support**.

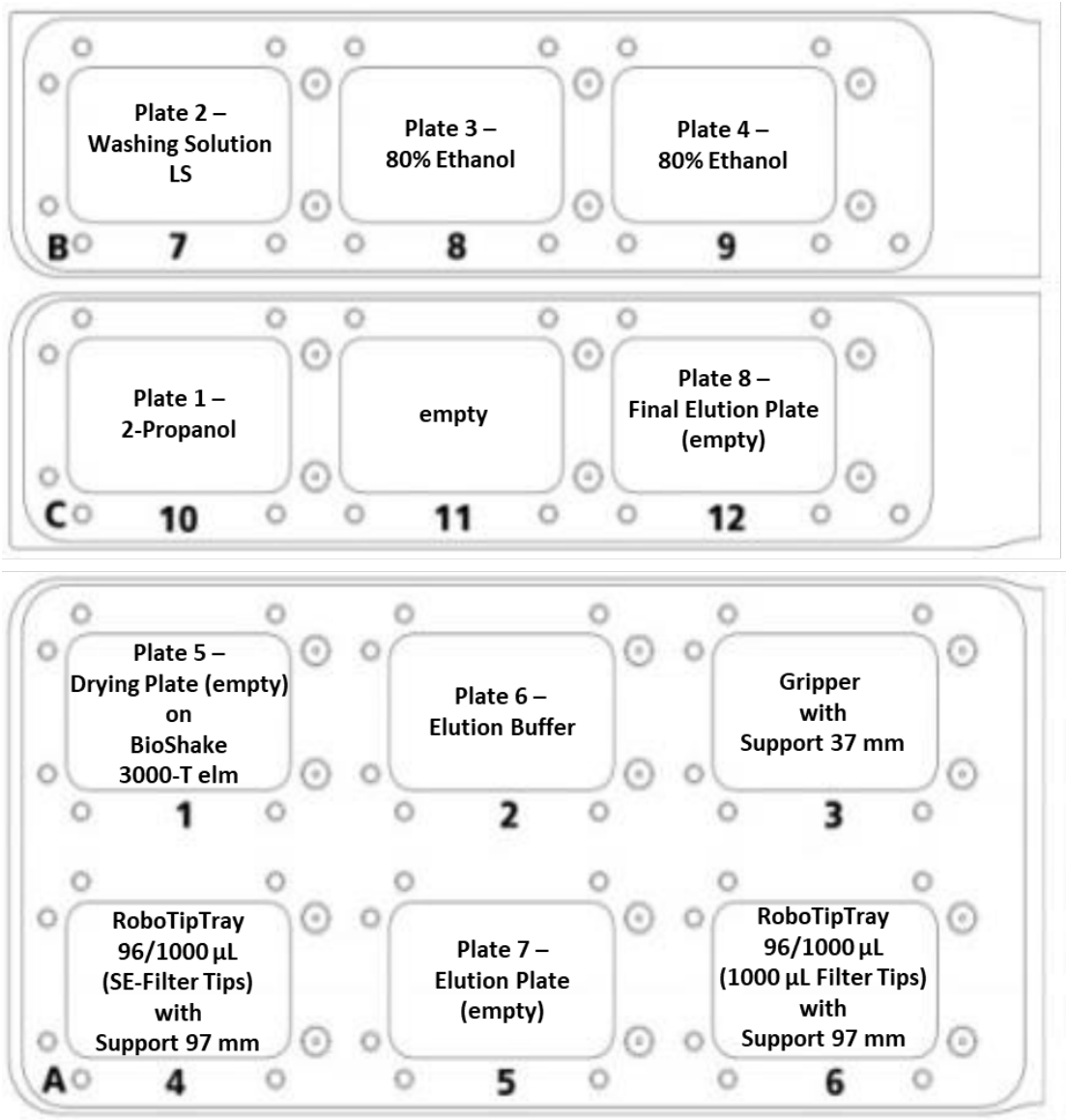


Figure 3: Deck layout for extraction.

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 need 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 4**).

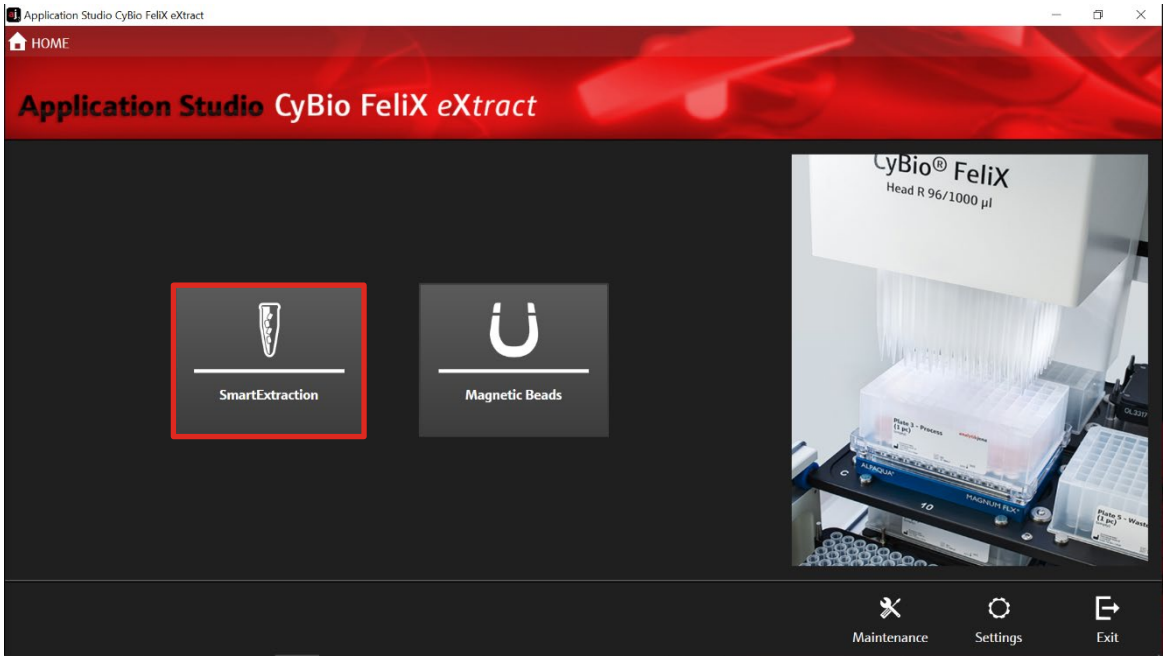


Figure 4: Homescreen of AppStudio FeliX *eXtract*. Selection of kit technology: SmartExtraction.

6. Select the extraction kit “smart DNA prep (a96) – FX, prefilled” (→ see **Figure 5**).

Figure 5: Selection of the extraction kit: smart DNA prep (a96) – FX, prefilled.

7. Check the right protocol version “External Lysis (a96) – 02” (→ see Figure 6).

Figure 6: Version number of the extraction protocol.

8. Adjust the “Elution Volume” to 150-500 µL according to the table below. Start the protocol by clicking the button “Execute” (→ see **Figure 7**).

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

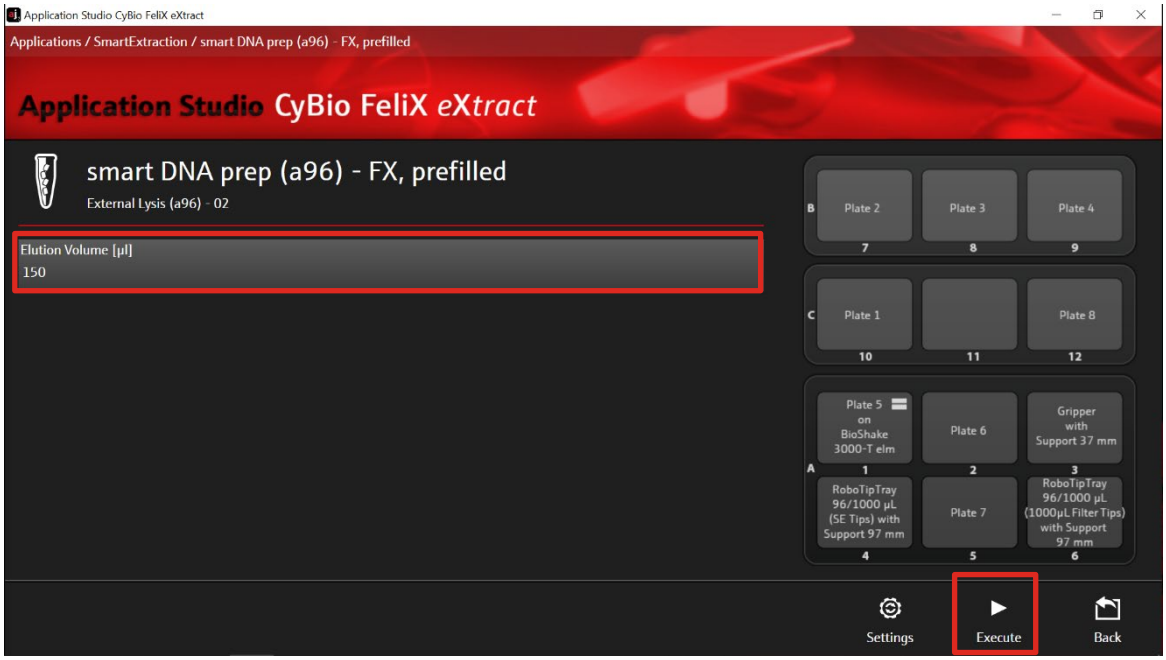


Figure 7: Adjustment of the elution volume.

9. After selecting “Execute” the deck layout is shown. Check the correct positioning of plates on corresponding deck positions and confirm with “Ok” (→ see **Figure 8**).

Figure 8: **Deck layout for checking the correct positions of all plates and accessories.**

10. 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 9**)

Figure 9: Purification process completed.

11. 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. 20) from the BioShake 3000-T-elm at **deck position 1**.

NOTE

When using alternate elution vessels (→ see section 10.3 “Loading the sample and starting CyBio FeliX”, p. 18), 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.

12. Afterwards, remove and discard the used Deep Well Plates and the used tips.

1 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 10.1 “Handling of SmartExtraction Pipette Tips” on p. 17).
High viscosity extracted DNA	
Insufficient amount of Elution Buffer	Elute the 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