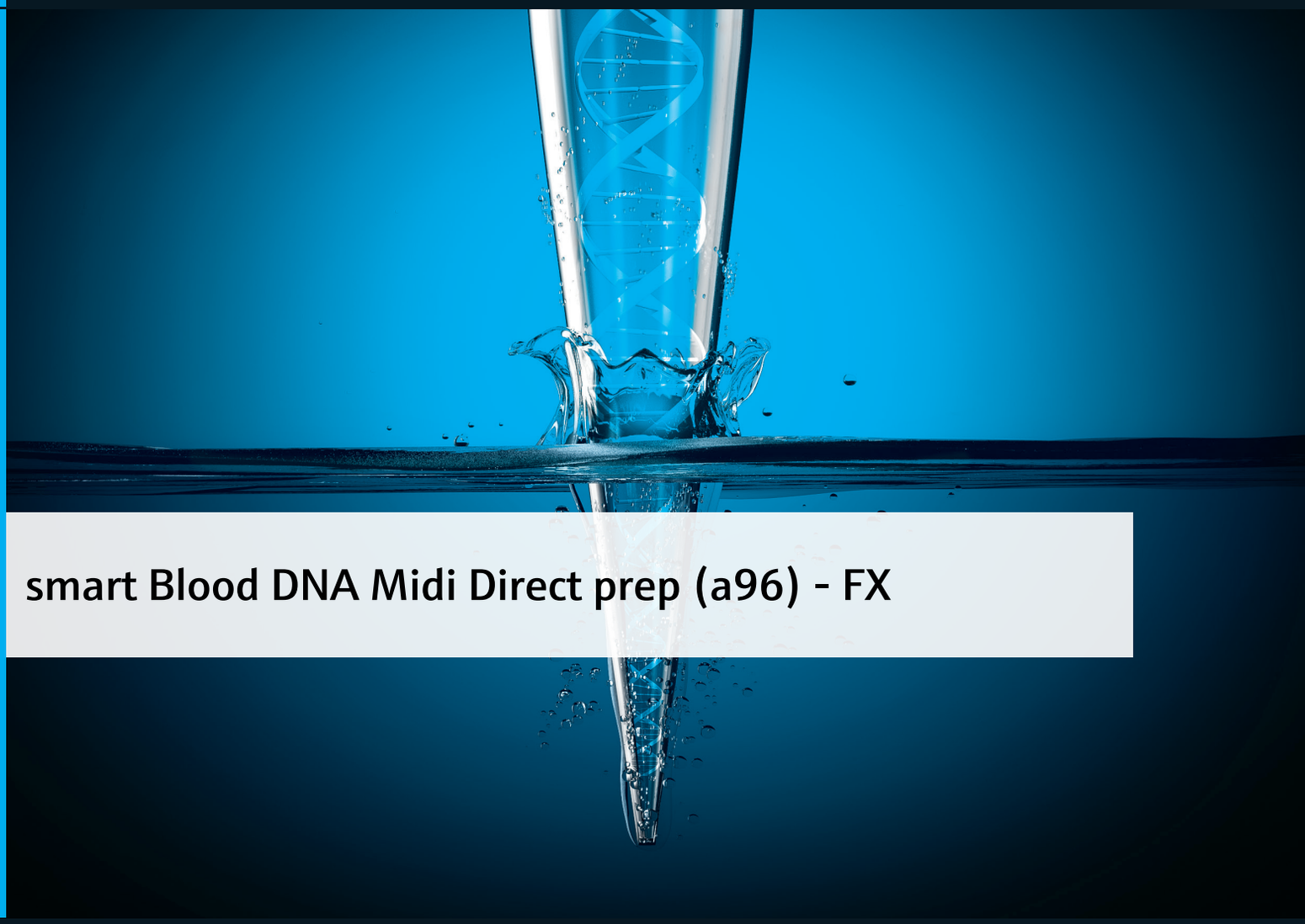
Instructions for Use
Life Science Kits & Assays



smart Blood DNA Midi Direct prep (a96) - FX

iST Innuscreen GmbH

Order No.:
845-FX-4296096 96 reactions
845-FX-4296480 480 reactions

Publication No.: HB_FX-4296_e_220602

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 **smart Blood DNA Midi Direct prep (a96) – FX** has been designed for automated isolation with CyBio FeliX of high molecular weight genomic DNA from whole blood samples stabilized with EDTA, citrate or heparin or sampled with PAXgene® Blood DNA Tubes. The kit utilizes the new SmartExtraction technology using Smart Modified Surfaces invented by IST Innuscreen GmbH (patent pending).

The extraction process is based on adsorption of the cells and genomic DNA to Smart Modified Surfaces inside a unique 1 mL filter tip in combination with CyBio FeliX. 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 simple 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 before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

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.
	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 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!

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 **smart Blood DNA Midi Direct 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 them 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 kit has been produced and tested in an ISO13485 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 Blood DNA Midi Direct 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 (→ see "Product specifications", p. 11). 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 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-4296096	845-FX-4296480
SmartExtraction Tips	6 x 16	30 x 16
Proteinase K	for 4 x 1.5 mL working solution	for 17 x 1.5 mL working solution
Buffer ERC	3 x 110 mL	15 x 110 mL
Lysis Solution CBO	50 mL	5 x 50 mL
Washing Solution LS (conc.)	15 mL	5 x 15 mL
Elution Buffer	60 mL	5 x 60 mL
Deep Well Plate (square, 2.0 mL)	10	50
Final Elution Plate	1	5
Sealing Foil	1	5
Protective Plate	2	10
Filter Tips	96	5 x 96
Manual	1	1

6.2 Components not included in the kit

- ddH_2O for dissolving **Proteinase K**
- 1.5 mL reaction tubes
- 80 % Ethanol (molecular biology grade, undenatured)
- 2-Propanol (molecular biology grade)
- 96 %–99.8 % Ethanol (molecular biology grade, undenatured)
- 1 column, 2 column and 4 column reservoirs for prefilling by CyBio FeliX (Smart Prefilling Set 2, 5x96 reactions, OL3317-25-129)
- RNase A (10 mg/mL) for RNA removal (if required)

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-8500115, 115 pcs, IST Innuscreen GmbH)
- Final Elution Plate (96 well, 1.2 mL) (31-01642, 5 pcs, IST Innuscreen GmbH)
- Smart Prefilling Set 2 (OL3317-25-129, 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

- 0.2–1.0 mL whole blood (fresh or frozen with a **maximum of two freeze/thaw cycles**) stabilized with EDTA, citrate, heparin or sampled with PAXgene® Blood DNA Tubes.
- 0.2–1.0 mL buffy coat (derived from up to 2.0 mL stabilized whole blood) generated with ATREUS or centrifugation. The blood has to fulfill the conditions described for extraction from whole blood as stated above.
-

NOTE

"Fresh" here means a maximum storage time of 24 hours at room temperature or maximum storage time of 6 days at 4 °C to 8 °C.

"Frozen" here means storage at -22 °C to -18 °C immediately after blood sampling.

Frozen starting materials stabilized with **Citrate-Phosphate-Derivative (CPD) is not suitable** for extraction with this kit.

2. Time for isolation and typical yield

External lysis steps are not required.

Sample volume	Automated prefilling	Extraction	Elution volume
0.5 mL	44 min	83 min	150-500 µL
1.0 mL	52 min	113 min	150-500 µL

3. Typical yield

Depends on amount and condition of starting material

Sample volume	Typical yield
0.5 ml	5–15 µg
1.0 ml	15–40 µg
Buffy coat (2 mL whole blood equivalent)	30–60 µg

NOTE

Yield of isolated DNA is affected by the condition of blood used. The condition of blood depends on storage conditions as well as the constitution of the donor. It must be considered that medical treatment of the donor may lower the yield of isolated DNA. This kit requires intact cells and may not work satisfyingly in case of damaged cells in the starting material!

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 the indicated amount of absolute ethanol to each bottle of **Washing Solution LS (conc.)** and mix thoroughly. Always keep the bottles firmly closed!

845-FX-4296096	Add 60 mL ethanol to 15 mL Washing Solution LS (conc.).
845-FX-4296480	Add 60 mL ethanol to 15 mL Washing Solution LS (conc.).

- Put accessories on the corresponding supports according to the following table:

Accessories	Support
CyBio RoboTipTray 1-96/1000 µL (OL3810-13-023)	Support; 97 mm height (OL3317-11-105)
Gripper (OL3317-11-800)	Support; 37 mm height (OL3317-11-120)
8-channel adapter Head R (OL3317-14-330)	Support; 37 mm height (OL3317-11-120)
Cover Magazine Head R (OL30-3316-200-11)	Support; 37 mm height (OL3317-11-120)

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

See Figure 1 in order to differentiate between CyBio RoboTipTray 1-96/1000 µL and CyBio TipRack 96/1000 µL.

Figure 1: Difference between CyBio RoboTipTray 1-96/1000 µL (left) and CyBio TipRack 96/1000 µL (right).

9 Prefilling of Reagent Plates for up to 500 µL sample volume

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

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**).

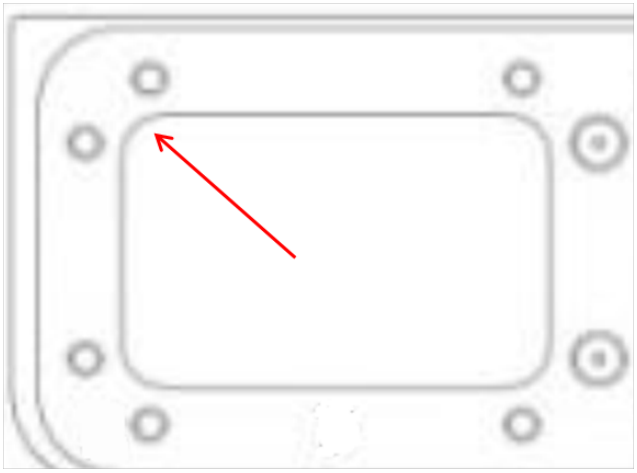


Figure 2: Positioning of plates and reservoirs on CyBio FeliX deck.

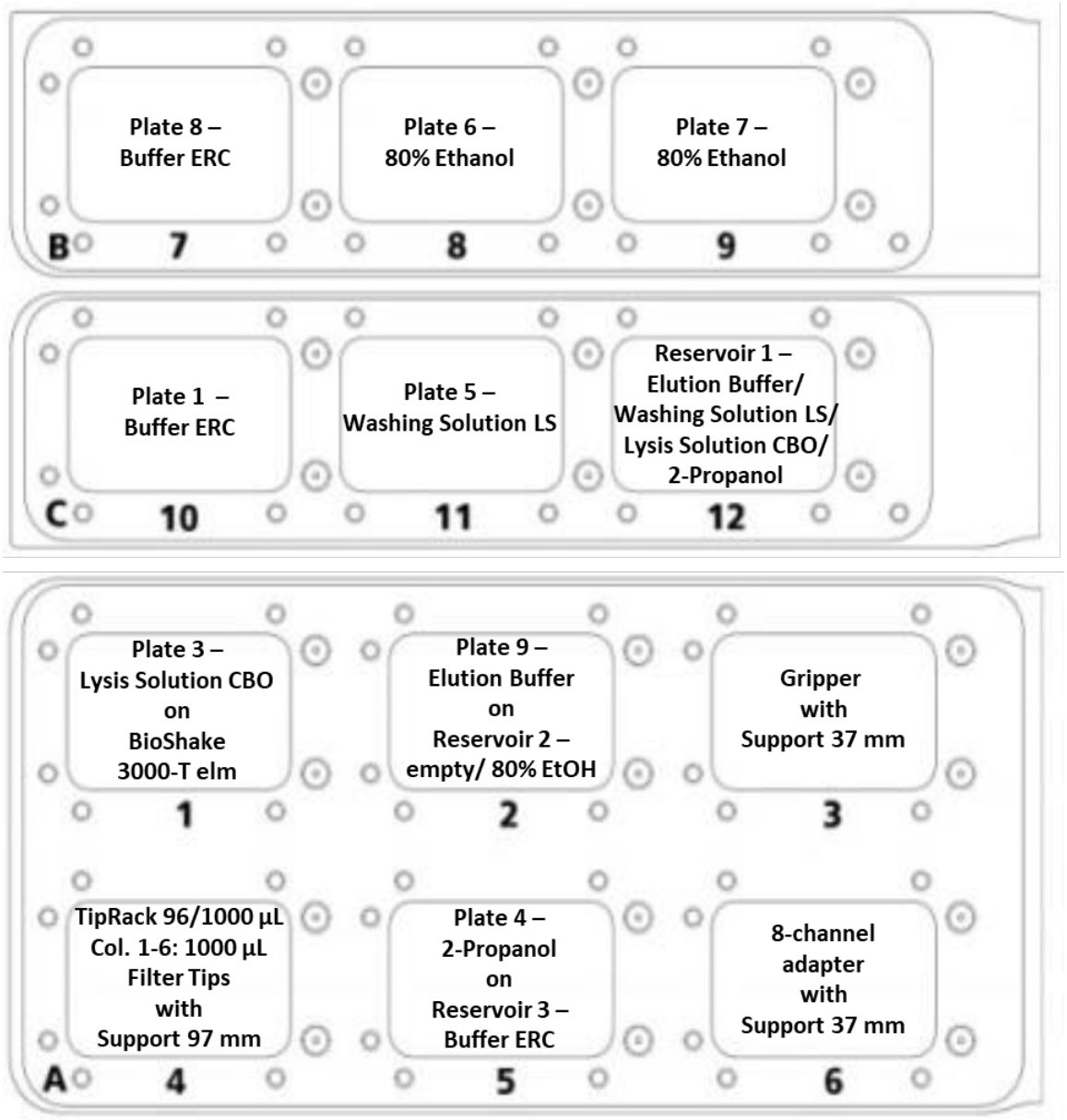


Figure 3: Deck Layout for the prefilling protocol for up to 500 µL sample volume.

NOTE

The prefilling is only recommended when prefilled plates are used immediately for the extraction process after prefilling.

1. Label the 1 column, 2 column and 4 column reservoirs from the Smart Prefilling Set 2 according to the table below:

Number	Label	
Reservoir 1 (4 column)	**Reservoir 1:**	
	Column 1	Elution Buffer
	Column 2	Washing Solution LS
	Column 3	Lysis Solution CBO
	Column 4	2-Propanol
Reservoir 2 (2 column)	**Reservoir 2:**	
	Left side of reservoir	empty
	Right side of reservoir	80 % Ethanol
Reservoir 3 (1 column)	**Reservoir 3:**	
	Buffer ERC	

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

Plate	Label
Plate 1	Buffer ERC
Plate 2*	
Plate 3	Lysis Solution CBO
Plate 4	2-Propanol
Plate 5	Washing Solution LS
Plate 6	80 % Ethanol
Plate 7	80 % Ethanol
Plate 8	Buffer ERC
Plate 9	Elution Buffer
Plate 10**	Elution Plate (empty)
Plate 11**	Final Elution Plate (empty)

* Not required for prefilling and extraction of 500 µL sample volume.
** Not required in the prefilling process, but for the extraction process. Put aside during prefilling.

3. Transfer the content of one bottle (60 mL) **Elution Buffer** into column 1 of the reservoir labeled “Reservoir 1 – Elution Buffer/ Washing Solution LS/ Lysis Solution CBO/ 2-Propanol”.
4. Transfer the content of one bottle (75 mL) **Washing Solution LS** into column 2 of the reservoir labeled “Reservoir 1 – Elution Buffer/ Washing Solution LS/ Lysis Solution CBO/ 2-Propanol”.
5. Transfer the content of one bottle (50 mL) **Lysis Solution CBO** into column 3 of the reservoir labeled “Reservoir 1 – Elution Buffer/ Washing Solution LS/ Lysis Solution CBO/ 2-Propanol”.
6. Transfer 40 mL **2-Propanol** into column 4 of the reservoir labeled “Reservoir 1 – Elution Buffer/ Washing Solution LS/ Lysis Solution CBO/ 2-Propanol”. Place the filled reservoir into the CyBio FeliX on position 12 (→ see **Figure 3**).
7. Transfer 120 mL **80 % Ethanol** into the **right** side of the 2 column reservoir labeled “Reservoir 2 – 80°% Ethanol”. Place the filled reservoir into the CyBio FeliX on position 2 (→ see **Figure 3**).
8. Transfer the content of two bottles (2 x 110 mL) **Buffer ERC** into the reservoir labeled “Reservoir 3 – Buffer ERC”. Place the filled reservoir into the CyBio FeliX on position 5 (→ see **Figure 3**).
9. Insert filter tips in columns 1-6 in the CyBio Tip Rack 96/1000 µL. Please fill the whole rows of the columns with filter tips.
10. Place the CyBio Tip Rack 96/1000 µL into the CyBio FeliX on position 4 (→ see **Figure 3**).
11. Place the 8-channel adapter (Head R 96) with the support 37 mm into the CyBio FeliX on position 6 (→ see **Figure 3**).
12. Place the empty, labeled plates on the CyBio FeliX deck according to the deck layout for up to 500 µL sample volume (→ see **Figure 3**).

NOTE
Please pay special attention to the following deck positions:
Position 1:
Place **Plate 3 – Lysis Solution CBO** directly on BioShake 3000-T elm on **position 1**.
Position 2: Place **Reservoir 2** directly on **position 2**. Stack **Plate 9 – Elution Buffer** on **Reservoir 2**.
Position 5: Place **Reservoir 3** directly on **position 5**. Stack **Plate 4 – 2-Propanol** on **Reservoir 3.**

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

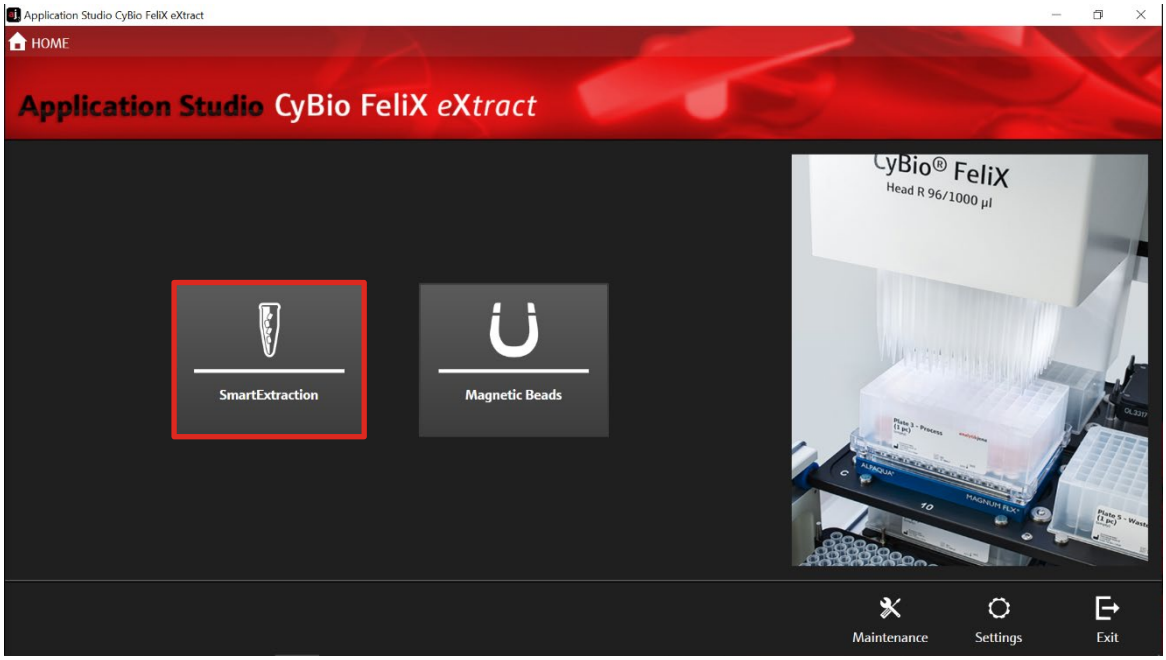


Figure 4: HomeScreen of the AppStudio FeliX eXtract. Selection of extraction technology: "SmartExtraction".

15. Choose "smart Blood DNA Midi Direct prep (a96) – FX" (→ see **Figure 5**).

Figure 5: Kit selection: smart Blood DNA Midi Direct prep (a96) - FX.

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

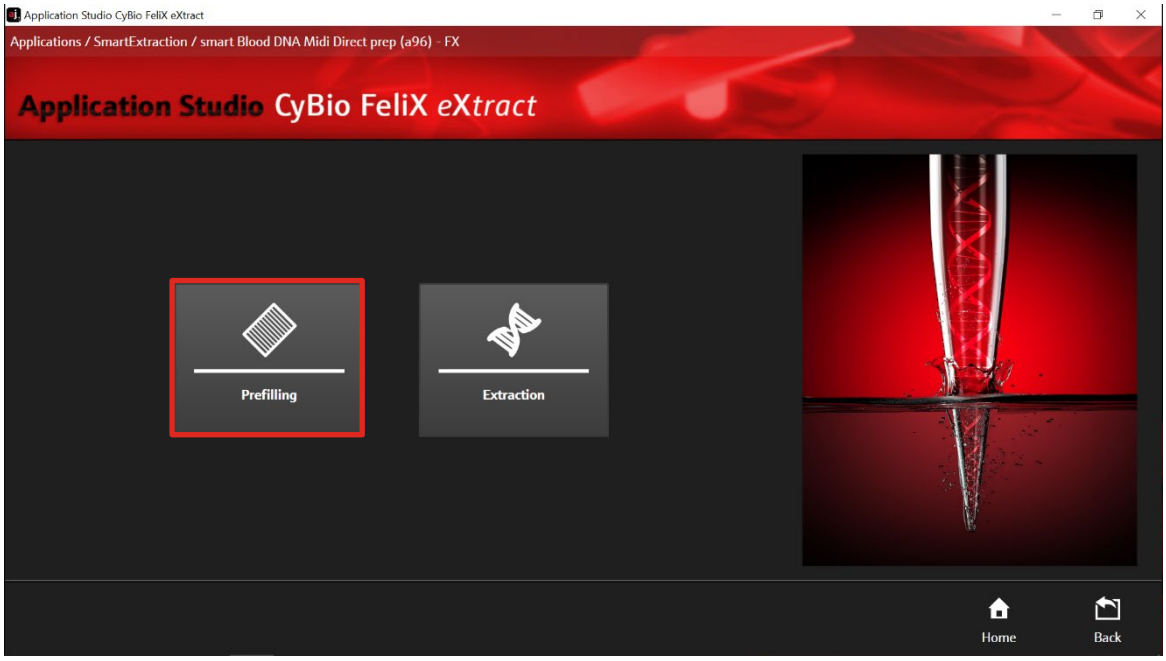


Figure 6: Routine selection: Prefilling.

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

18. Check the correct version number of the protocol “Prefilling – SE Internal Lysis (a96) – 01”. (→ see **Figure 7**)

Figure 7: Version number of the prefilling protocol.

19. To adjust the sample volume, click the button “Protocol” and select “500” for up to 500 µL sample volume (→ see Figure 8).

Figure 8: Selection of prefilling protocol for up to 500 µL sample volume.

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

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

22. 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 10**).

Figure 10: Completion of the prefilling routine.

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. Discard the reservoirs and all their contents.
26. The plates **Plate 1 – Buffer ERC**, **Plate 6 – 80% Ethanol**, **Plate 7 – 80% Ethanol** and the **Gripper with Support 37 mm** do not have to be removed for the extraction process.

9.2 Manual prefilling of Deep Well Plates

NOTE

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	Volume per well
Plate 1	Buffer ERC	**1050 µL** Buffer ERC
Plate 2**		
Plate 3	Lysis Solution CBO	**400 µL** Lysis Solution CBO
Plate 4	2-Propanol	**350 µL** 2-Propanol
Plate 5	Washing Solution LS	**600 µL** Washing Solution LS
Plate 6	80 % Ethanol	**600 µL** 80 % Ethanol
Plate 7	80 % Ethanol	**600 µL** 80 % Ethanol
Plate 8	Buffer ERC	**1050 µL** Buffer ERC
Plate 9	Elution Buffer	**600 µL** Elution Buffer
Plate 10*	Elution Plate	**empty**
Plate 11*	Final Elution Plate	**empty**

* Not required in the prefilling process, but for the extraction process. Put aside during prefilling.
**If you are using the 200-500 µL sample volume, Plate 2 is not required for the extraction process.

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.

10 Sample preparation of up to 500 µL whole blood

10.1 Preparation of buffy coat from whole blood

1. Transfer up to 2.0 mL whole blood into a 2.0 mL tube.
2. Centrifuge for 10 minutes with 2,500 x g at 4 °C.
3. Carefully aspirate and discard the transparent upper layer. Do not disturb the interphase!
4. Carefully aspirate the interphase and transfer into a new 1.5 mL tube.

10.2 Preparation of Reagent Plates for up to 500 µL sample volume

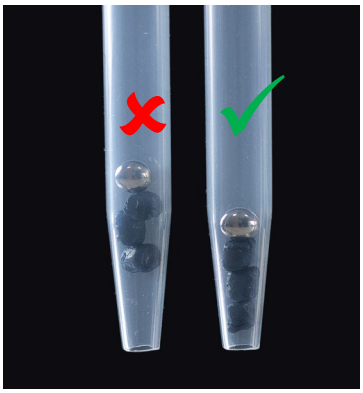
1. Add **50 µL Proteinase K** into cavities of **Plate 3 – Lysis Solution CBO** according to your sample layout.
2. Transfer blood sample or buffy coat and water according to the following table into the cavities of **Plate 1 – Buffer ERC**. First, transfer the blood sample or buffy coat and then transfer the water.

Sample volume	Volume of sterile water
0.2 mL	0.3 mL
0.3 mL	0.2 mL
0.4 mL	0.1 mL
0.5 mL	-

11 Automated extraction of up to 500 µL sample volume

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 the SmartExtraction Tips.
Make sure that the Smart Modified Material is collected near the outlet of the Smart-Extraction Tip. If necessary, invert the tip a few times or flick the tip 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 11.

Figure 11: Checking SmartExtraction Tips.

11.2 Loading of CyBio FeliX

The smart Blood DNA Midi Direct prep (a96) – FX is optimized for sample volumes ranging from 200–1000 µL of whole blood. Due to the wide volume range, preparation differs for smaller (200 – 500 µL) and larger (600 – 1000 µL) sample volumes (→ see sections 10.2 and 13.2, respectively).

1. Load all prepared plates and accessories onto CyBio FeliX decks according to **Figure 12**. As a Final Elution Plate (**position 12**) multiple options are possible:

   - Plate 11 - 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 3 – Lysis Solution CBO** on the BioShake 3000-T-elm (**deck position 1**).
Position 2: Place **Plate 9 – Elution Buffer** directly on **position 2**. Stack **Plate 4 – 2-Propanol** on **Plate 9 – Elution Buffer**.
Position 5: Place **Plate 10 – Elution Plate (empty)** directly on **position 5**. Stack **Plate 8 – Buffer ERC** on **Plate 10 – Elution Plate (empty).**
Position 4 and 6: Put the **Protective Plate** directly on the bottom of the **97 mm support!**

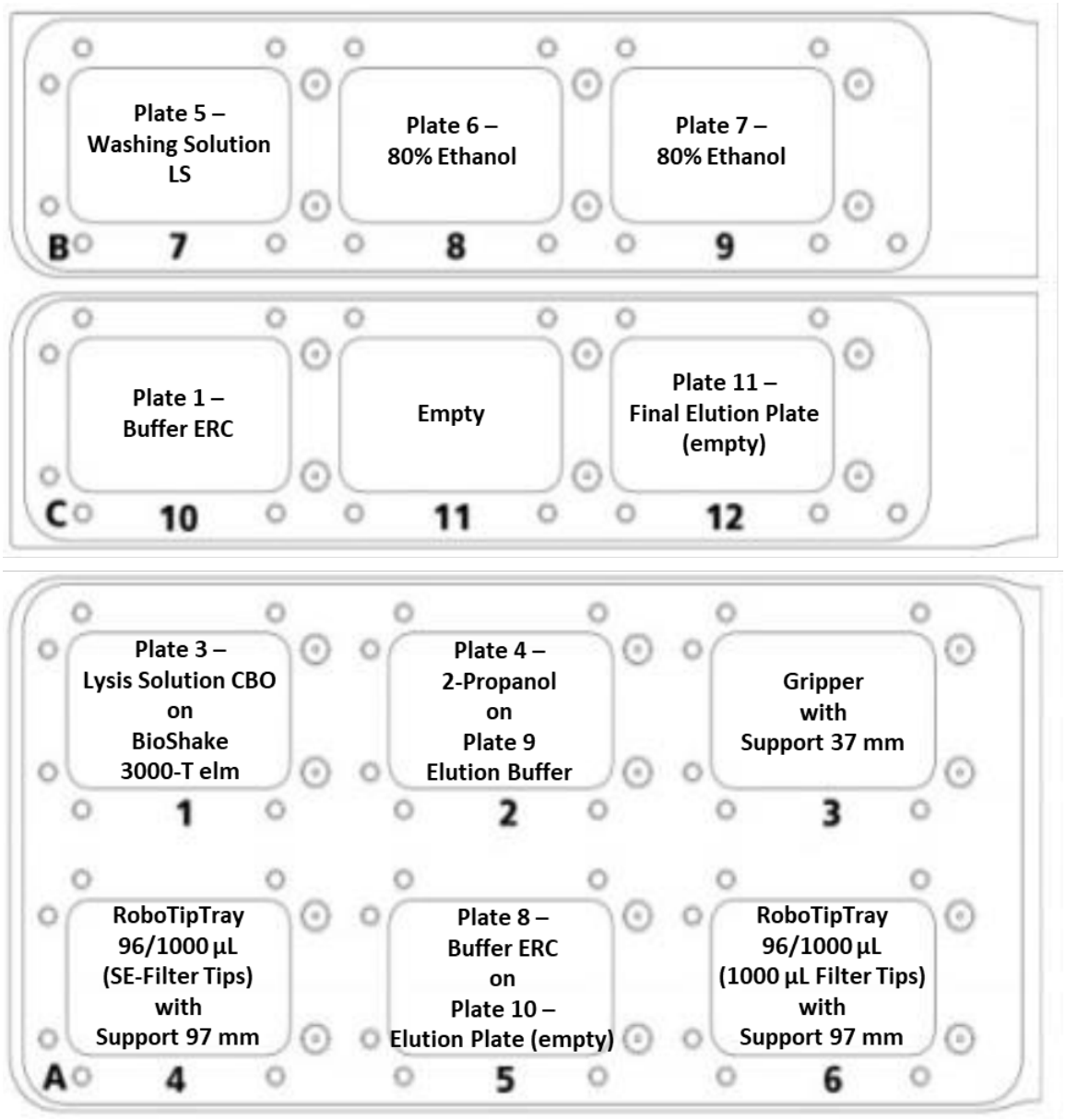


Figure 12: Deck layout for the extraction of 200–500 µL sample material.

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 10 – Elution Plate** at the end of the protocol. In this case, **Plate 11 – Final Elution Plate** does not need to be placed on **deck position 12**. Transfer of the eluate into storage tubes has to be done manually. In order to avoid a loss of DNA integrity pipet carefully with a wide-bore or cut tip.

2. Switch on CyBio FeliX and open the AppStudio FeliX *eXtract*.
3. Select "SmartExtraction" (→ see **Figure 13**).

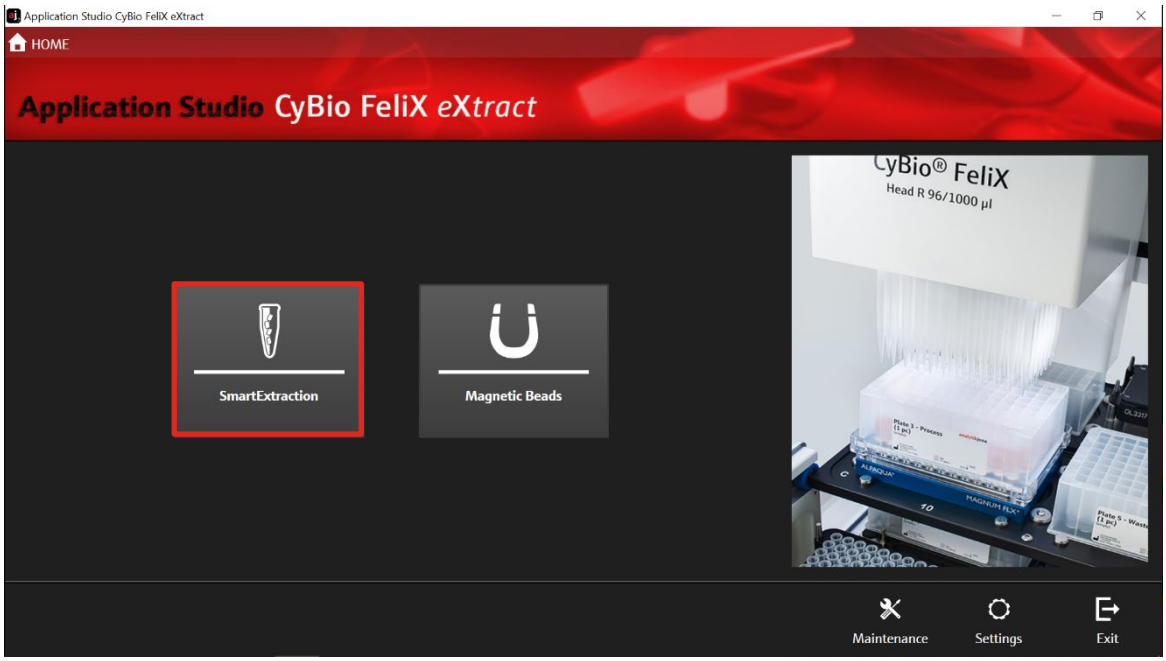


Figure 13: Homescreen AppStudio FeliX eXtract. Selection of extraction technology: SmartExtraction.

4. Select the kit protocol "smart Blood DNA Midi Direct prep (a96) – FX" (→ see **Figure 14**).

Figure 14: Selection of extraction kit: smart Blood DNA Midi Direct prep (a96) – FX.

5. Select “Extraction” (→ see **Figure 15**).

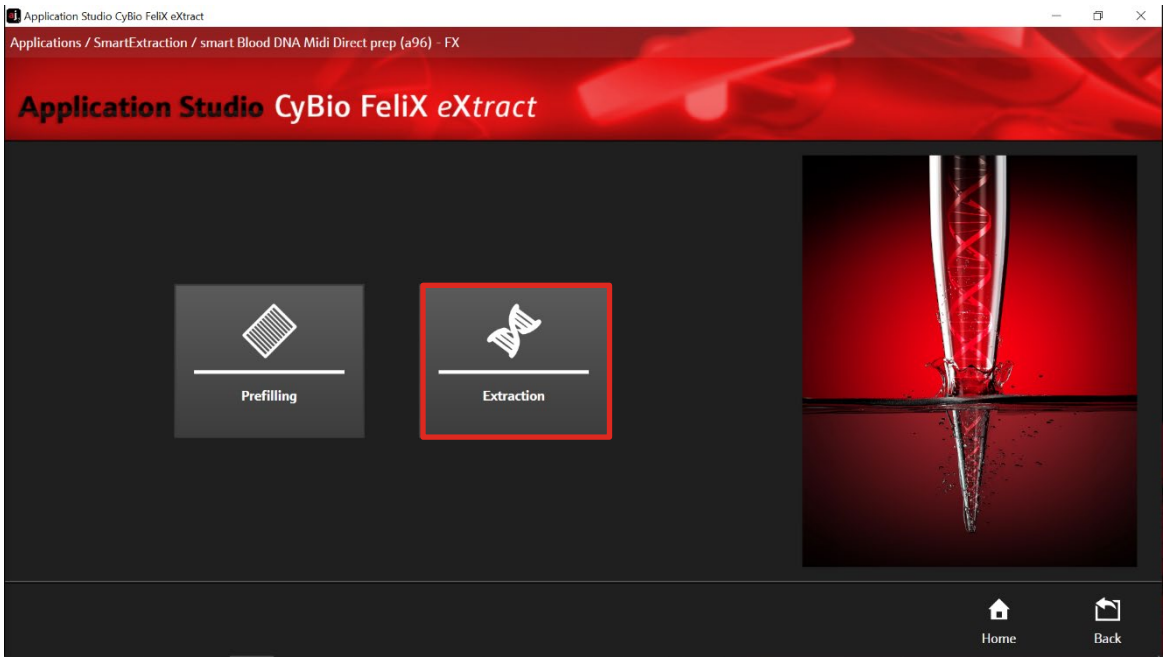


Figure 15: Routine selection: Extraction.

6. After selecting “Extraction” the Extraction Start Screen appears (→ see **Figure 17**).
7. Check the correct protocol version “Internal Lysis (a96) – 02” (→ see **Figure 16**).

Figure 16: Version number of the extraction protocol.

8. Select the protocol by choosing the corresponding sample volume (500 µL) sample volume and adjust the "Elution Volume" between **150 – 500 µL** using the text field (→ see **Figure 17**). Start the protocol by clicking the button "Execute".

Figure 17: Selection of sample volume (500 µL) and elution volume (variable).

9. Check the correct positioning of plates and accessories on the corresponding deck positions and confirm with "Ok" (→ see Figure 18).

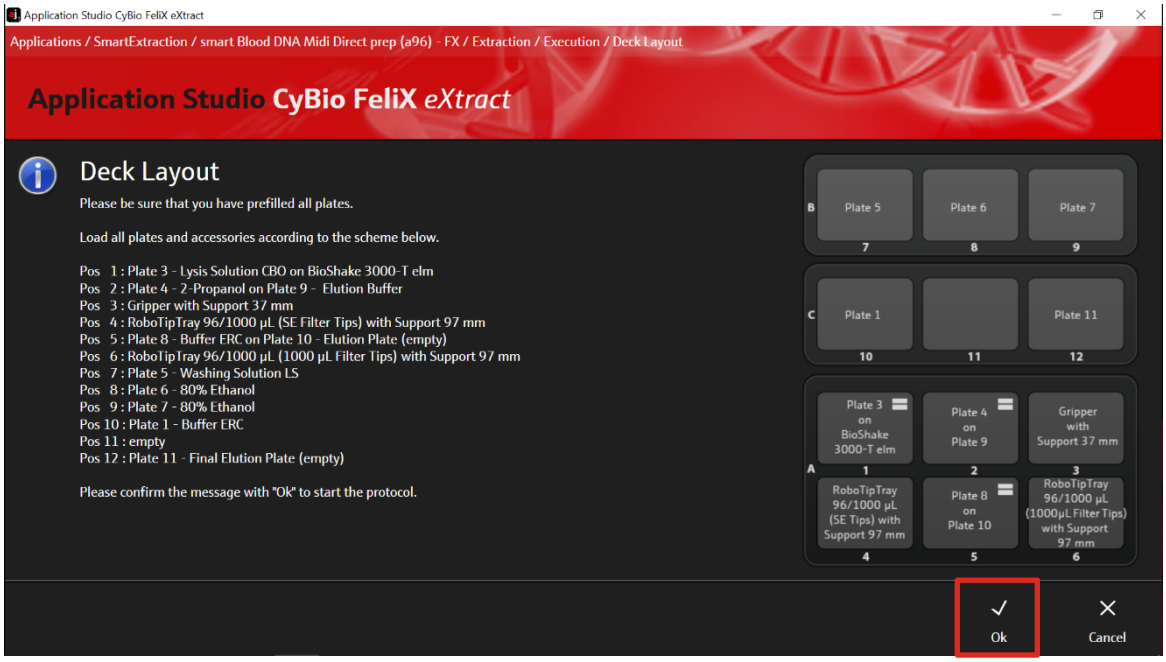


Figure 18: List of deck positions and corresponding plates.

8. The chosen protocol is performed by the device. After the protocol is finished, the message “Purification process completed” is shown on the screen of the computer. Confirm the message with “Ok” (→ see Figure 19).

Figure 19: Purification process completed.

9. Once the extraction is completed, remove **Plate 11 – Final Elution Plate** from **deck position 12** or **Plate 10 – Elution Plate** (→ see Note on p. 25) from the BioShake 3000-T-elm at **deck position 1**.

10. Seal the respective plate with the included sealing film and store DNA under adequate conditions.

NOTE
When using alternate elution vessels as listed in "Loading of CyBio FeliX" on page 23), proceed analogously.
Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C. For long-term storage we recommend -80 °C.

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

12 Prefilling of Reagent Plates for up to 1000 µL sample volume

There is the option to prefill the plates automatically with the CyBio FeliX (→ see section 12.1) or manually (→ see section 12.2).

12.1 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 20**).

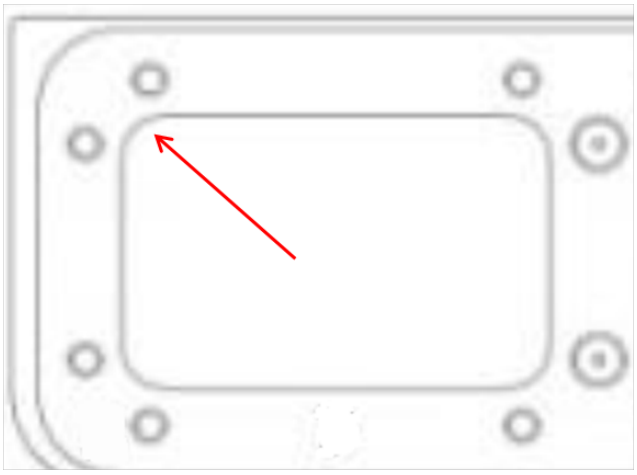


Figure 20: Positioning of plates and reservoirs on CyBio FeliX deck.

NOTE
The prefilling is only recommended when prefilled plates are used immediately for the extraction process after prefilling.

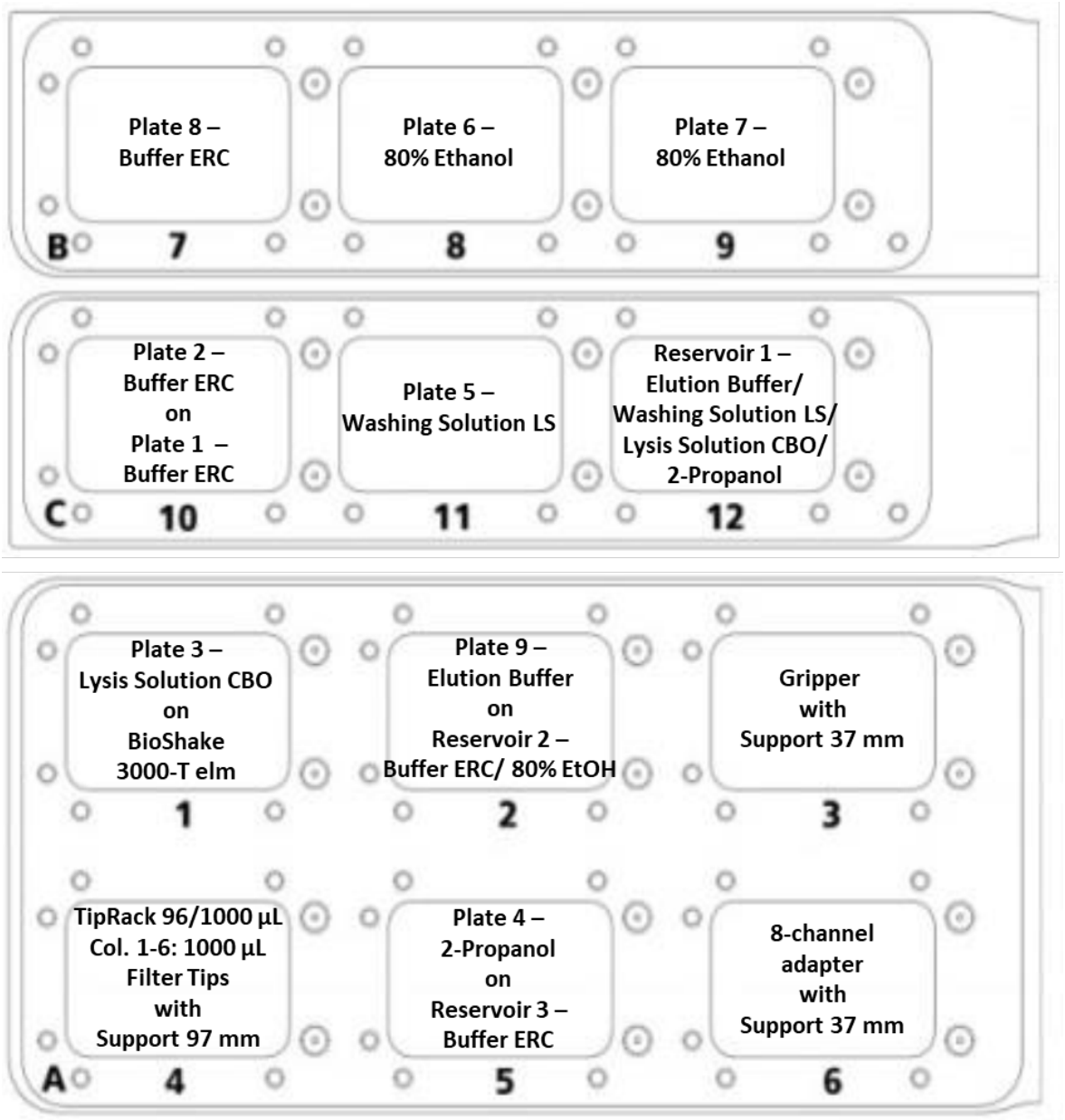


Figure 21: Deck layout for the prefilling protocol for 600 to 1000 µL sample volume.

1. Label the 1 column, 2 column and 4 column reservoirs from the smart Prefilling Set 2 according to the table below:

Number	Label	
Reservoir 1 (4 column)	**Reservoir 1:** Column 1 Column 2 Column 3 Column 4	 Elution Buffer Washing Solution LS Lysis Solution CBO 2-Propanol
Reservoir 2 (2 column)	**Reservoir 2:** Left side of reservoir Right side of reservoir	 Buffer ERC 80 % Ethanol
Reservoir 3 (1 column)	**Reservoir 3:** Buffer ERC	

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

Plate	Label
Plate 1	Buffer ERC
Plate 2	Buffer ERC
Plate 3	Lysis Solution CBO
Plate 4	2-Propanol
Plate 5	Washing Solution LS
Plate 6	80 % Ethanol
Plate 7	80 % Ethanol
Plate 8	Buffer ERC
Plate 9	Elution Buffer
Plate 10*	Elution Plate (empty)
Plate 11*	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 (60 mL) **Elution Buffer** into column 1 of the reservoir labeled “Reservoir 1 – Elution Buffer/ Washing Solution LS/ Lysis Solution CBO/ 2-Propanol”.
4. Transfer the content of one bottle (60 mL) **Washing Solution LS** into column 2 of the reservoir labeled “Reservoir 1 – Elution Buffer/ Washing Solution LS/ Lysis Solution CBO/ 2-Propanol”.
5. Transfer the content of one bottle (50 mL) **Lysis Solution CBO** into column 3 of the reservoir labeled “Reservoir 1 – Elution Buffer/ Washing Solution LS/ Lysis Solution CBO/ 2-Propanol”.
6. Transfer 40 mL **2-Propanol** into column 4 of the reservoir labeled “Reservoir 1 - Elution Buffer/ Washing Solution LS/ Lysis Solution CBO/ 2-Propanol”. Place the filled reservoir into the CyBio FeliX on position 12 (→ see **Figure 21**).
7. Transfer the content of one bottle (110 mL) **Buffer ERC** into the **left** side of the 2 column reservoir labeled “Reservoir 2 – Buffer ERC/ 80°% Ethanol”.
8. Transfer 120 mL **80% Ethanol** into the **right** side of the 2 column reservoir labeled “Reservoir 2 – Buffer ERC/ 80°% Ethanol”. Place the filled reservoir into the CyBio FeliX on position 2 (→ see **Figure 21**).
9. Transfer the content of two bottles (2 x 110 mL) **Buffer ERC** into the reservoir labeled “Reservoir 3 – Buffer ERC”. Place the filled reservoir into the CyBio FeliX on position 5 (→ see **Figure 21**).
10. Insert filter tips in columns 1-6 in the CyBio Tip Rack 96/1000 µL. Please fill the whole rows of the columns with filter tips.
11. Place the CyBio TipRack 96/1000 µL into the CyBio FeliX on position 4 (→ see **Figure 21**).
12. Place the 8-channel adapter (Head R 96) with the support 37 mm into the CyBio FeliX on position 6 (→ see **Figure 21**).
13. Place the empty, labeled plates on the CyBio FeliX deck according to the deck layout for the prefilling protocol for up to 1000 µL sample volume (→ see **Figure 21**).

NOTE
Please pay special attention to the following deck positions:

Position 1:
Place **Plate 3 – Lysis Solution CBO** directly on BioShake 3000-T elm on position 1.
Position 2: Place **Reservoir 2** directly on **position 2**. Stack **Plate 9 – Elution Buffer** on **Reservoir 2**.
Position 5: Place **Reservoir 3** directly on **position 5**. Stack **Plate 4 – 2-Propanol** on **Reservoir 3.**
Position 10: Place **Plate 1 – Buffer ERC** directly on **position 10.** Stack **Plate 2 – Buffer ERC** on **Plate 1 – Buffer ERC.**

14. Switch on the CyBio FeliX and open AppStudio FeliX *eXtract*.
15. Select the extraction technology "SmartExtraction" (→ see **Figure 22**) and the extraction kit "smart Blood DNA Midi direct prep (a96) – FX" (→ see **Figure 23**).

Figure 22: HomeScreen of the AppStudio FeliX eXtract. Selection of extraction technology: "SmartExtraction".

Figure 23: Kit selection: smart Blood DNA Midi Direct prep (a96) - FX.

16. Choose “Prefilling” (→ see **Figure 24**).

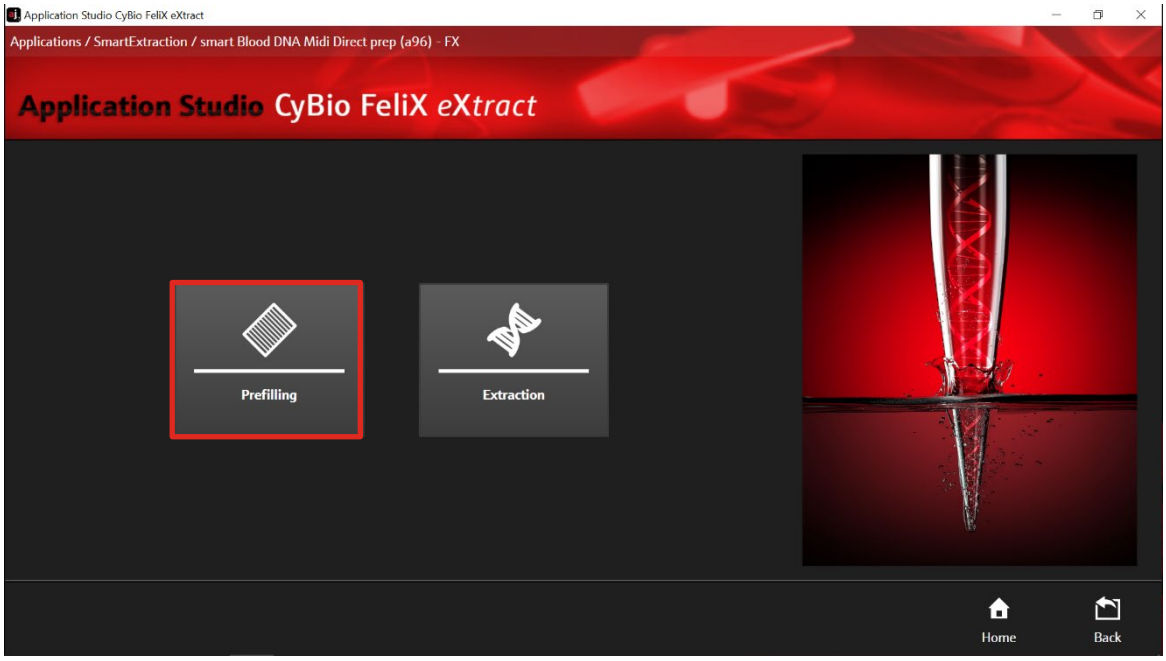


Figure 24: Routine selection: Prefilling.

17. After selecting “Prefilling” the Prefilling Start Screen appears (→ see **Figure 26**).

18. Check the correct version number of the protocol (→ see **Figure 25**) “Prefilling – SE Internal Lysis (a96) – 01”.

Figure 25: Version number of the prefilling protocol.

19. To adjust the sample volume, click the button “Protocol”. Choose “1000 µL” for sample volumes between 600 and 1000 µL (→ see **Figure 26**).

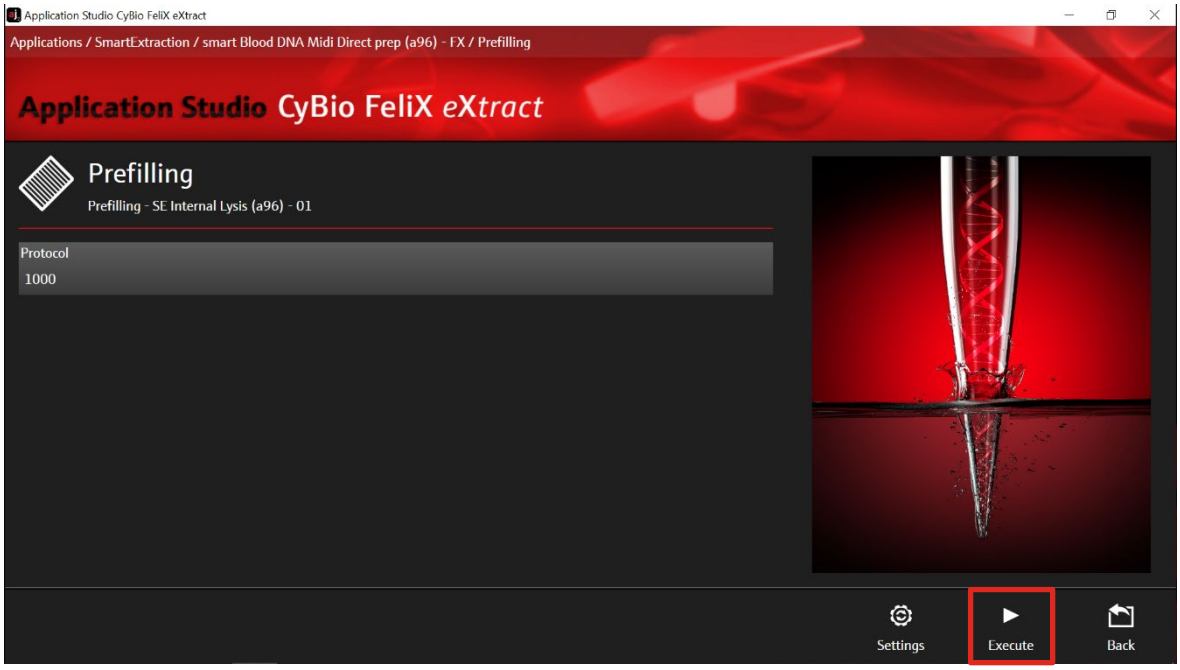


Figure 26: Selection of the prefilling protocol for up to 1000 µL sample volume.

20. Check the chosen parameters and confirm with “Execute”.
21. Check the correct deck positions of all plates, reservoirs and other hardware components (compare with list displayed in AppStudio FeliX *eXtract*, → see **Figure 27**) and confirm with “Ok”.

Figure 27: Deck layout for checking the right positions of all plates and accessories.

22. 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 28**).

Figure 28: Prefilling process is 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. Discard the reservoirs and all their contents.
26. The plates **Plate 1- Buffer ERC**, **Plate 6 – 80% Ethanol**, **Plate 7 – 80% Ethanol** and the **Gripper with Support 37 mm** do not have to be removed for the extraction process.

12.2 Manual prefilling of Deep Well Plates

NOTE

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 required wells of the Deep Well Plates according to the table below.

Plate	Label	Volume per well
Plate 1	Buffer ERC	**1050 µL** Buffer ERC
Plate 2**	Buffer ERC	**1050 µL** Buffer ERC
Plate 3	Lysis Solution CBO	**400 µL** Lysis Solution CBO
Plate 4	2-Propanol	**350 µL** 2-Propanol
Plate 5	Washing Solution LS	**600 µL** Washing Solution LS
Plate 6	80 % Ethanol	**600 µL** 80 % Ethanol
Plate 7	80 % Ethanol	**600 µL** 80 % Ethanol
Plate 8	Buffer ERC	**1050 µL** Buffer ERC
Plate 9	Elution Buffer	**600 µL** Elution Buffer
Plate 10*	Elution Plate	**empty**
Plate 11*	Final Elution Plate	**empty**

* Not required in the prefilling process, but for the extraction process. Put aside during prefilling.
**If you are using the 200-500 µL sample volume you do not have to prefill Plate 2.

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.

13 Sample preparation of up to 1000 µL sample volume

13.1 Preparation of buffy coat from whole blood

1. Transfer up to 2.0 mL whole blood into a 2.0 mL tube.
2. Centrifuge for 10 minutes with 2,500 x g at 4 °C.
3. Carefully aspirate and discard the transparent upper layer. Do not disturb the interphase!
4. Carefully aspirate the interphase and transfer into a new 1.5 mL tube.

13.2 Preparation of Reagent Plates for up to 1000 µL sample volume

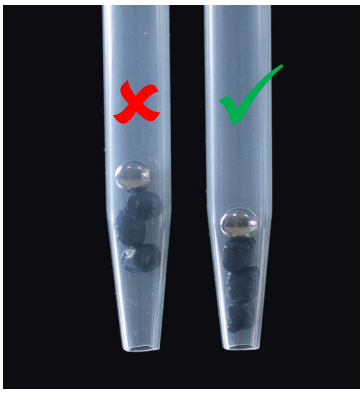
1. Add **50 µL Proteinase K** into appropriate cavities of **Plate 3 – Lysis Solution CBO**.
2. Transfer blood sample or buffy coat and water according to the following table into the cavities of **Plate 1 – Buffer ERC** and **Plate 2 – Buffer ERC**. Transfer the blood sample or buffy coat first and then add water.

Total sample volume	Sample volume per plate	Volume of sterile water per plate
0.6 mL	0.3 mL in Plates 1 and 2	0.2 mL in Plate 1 and 2
0.7 mL	0.35 mL in Plates 1 and 2	0.15 mL in Plate 1 and 2
0.8 mL	0.4 mL in Plates 1 and 2	0.1 mL in Plate 1 and 2
0.9 mL	0.45 mL in Plates 1 and 2	0.05 mL in Plate 1 and 2
1.0 mL	0.5 mL in Plates 1 and 2	-

14 Automated extraction of up to 1000 µL sample volume

14.1 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 the tip 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 29**.

Figure 29: Checking SmartExtraction Tips.

14.2 Loading of CyBio FeliX

The smart Blood DNA Midi Direct prep (a96) – FX is optimized for sample volumes ranging from 200–1000 µL of whole blood. Due to the wide volume range, preparation differs for smaller (200 – 500 µL) and larger (600 – 1000 µL) sample volumes (→ see sections 10.2 and 13.2, respectively).

1. Load all plates and accessories onto CyBio FeliX decks according to **Figure 30**. As a final Elution Plate (**position 12**) multiple options are possible:

   - **Plate 11 - 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 3 – Lysis Solution CBO** on the BioShake 3000-T-elm (**deck position 1**).
Position 2: Place **Plate 9 – Elution Buffer** directly on **position 2**. Stack **Plate 4 – 2-Propanol** on **Plate 9 – Elution Buffer**.
Position 5: Place **Plate 10 – Elution Plate (empty)** directly on **position 5**. Stack **Plate 8 – Buffer ERC** on **Plate 10 – Elution Plate (empty)**.
Position 4 and 6: Put the **Protective Plate** directly on the bottom of the **97 mm support**!

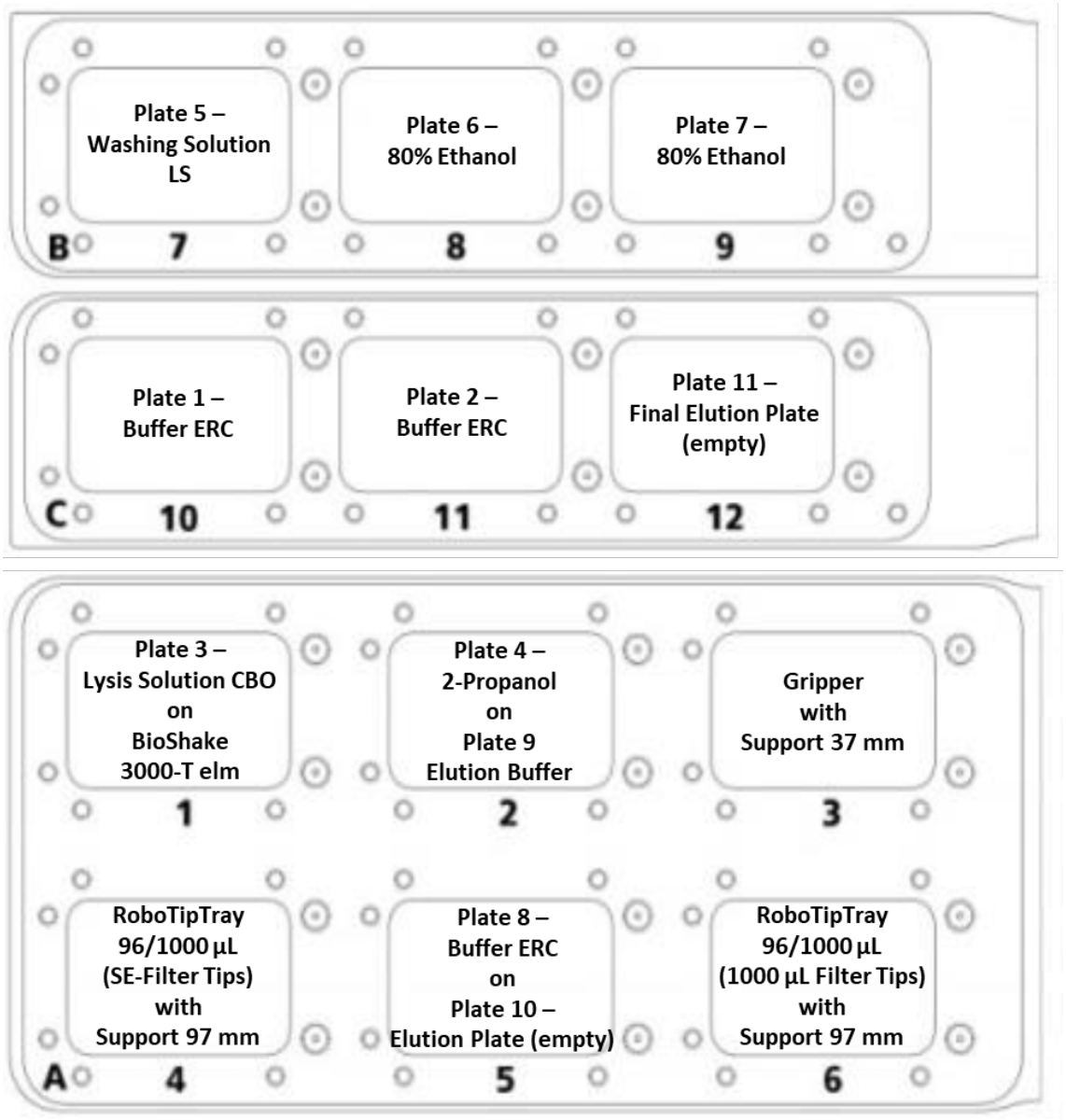


Figure 30: Deck layout for the extraction from 600–1000 µL sample material.

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 RoboTip Tray** 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 10 – Elution Plate** at the end of the protocol. In this case, **Plate 11 – 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 or 1.5 mL reaction tubes) has to be done manually. In order to avoid loss of DNA integrity pipet carefully with a wide-bore or cut tip.

2. Switch on CyBio FeliX and open the AppStudio FeliX *eXtract*.
3. Select the extraction technology “SmartExtraction” (→ see **Figure 31**).

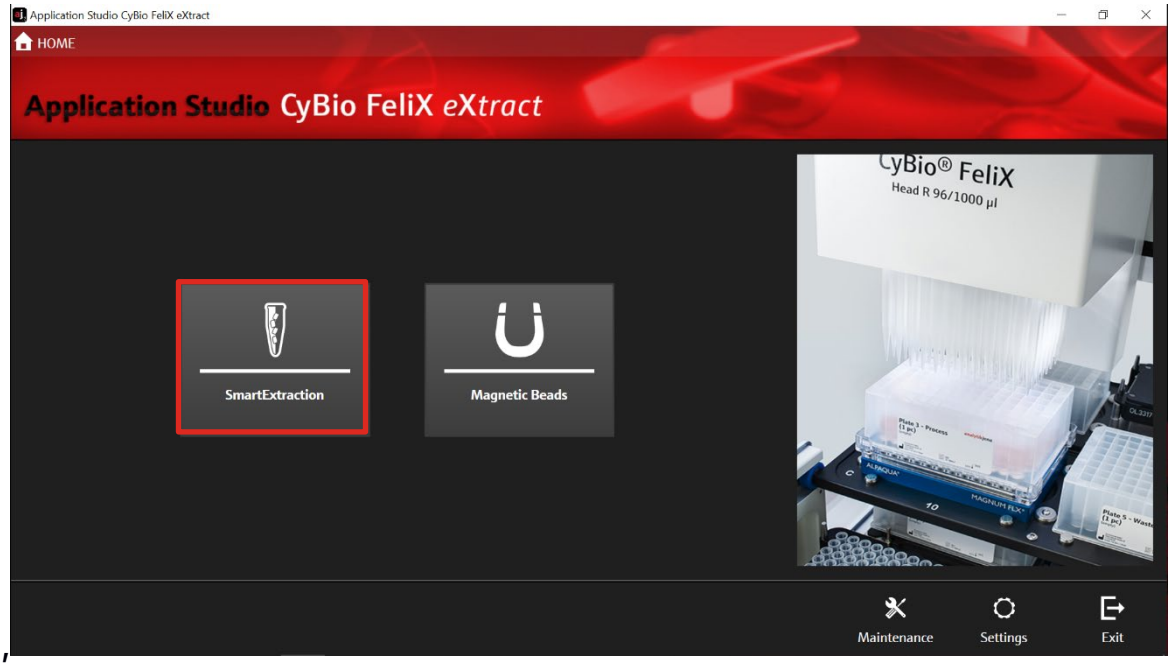


Figure 31: Homescreen of the AppStudio FeliX eXtract. Selection of extraction technology: SmartExtraction.

4. Select the kit protocol “smart Blood DNA Midi Direct prep (a96) – FX” (→ see **Figure 32**).

Figure 32: Selection of extraction kit: smart Blood DNA Midi Direct prep (a96) - FX.

5. Select “Extraction” (→ see **Figure 33**).

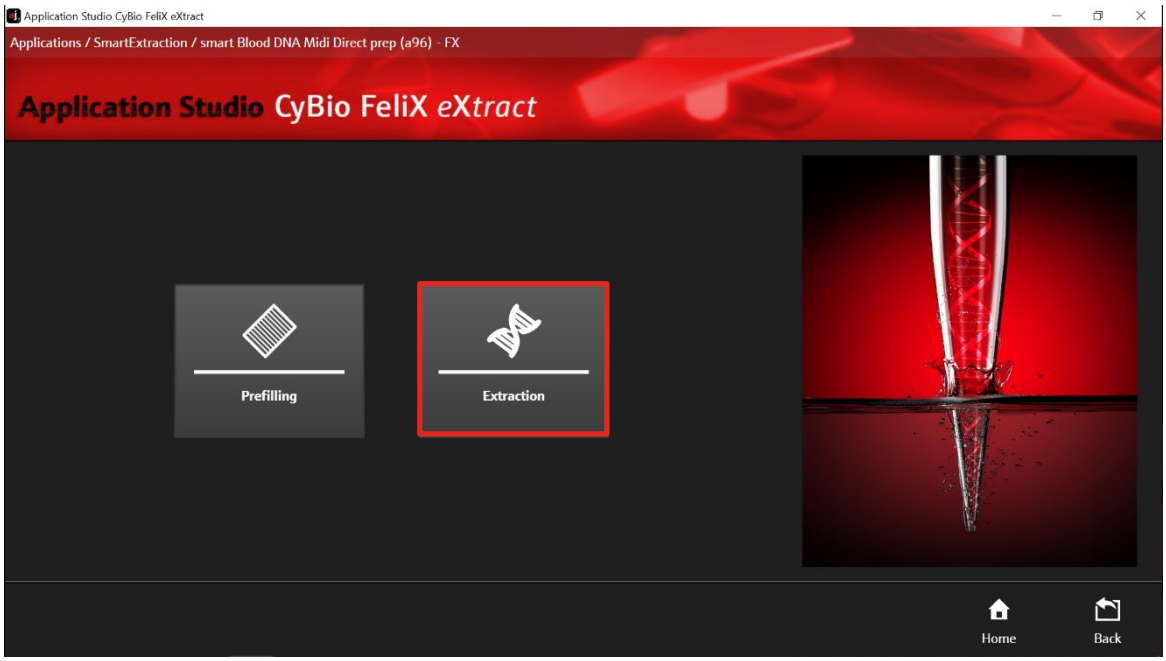


Figure 33: Routine selection: Extraction.

6. After selecting “Extraction”, the Extraction Start Screen appears (→ see **Figure 35**).
7. Check the correct protocol version “Internal Lysis (a96) – 02” (→ see **Figure 34**).

Figure 34: Version number of extraction protocol.

8. Select the protocol for 1000 µL sample volume and adjust the elution volume between **150-500 µL** (→ see **Figure 35**). Start the protocol by clicking “Execute”.

Figure 35: Selection of sample volume (1000 µL) and elution volume (variable).

9. 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 36**).

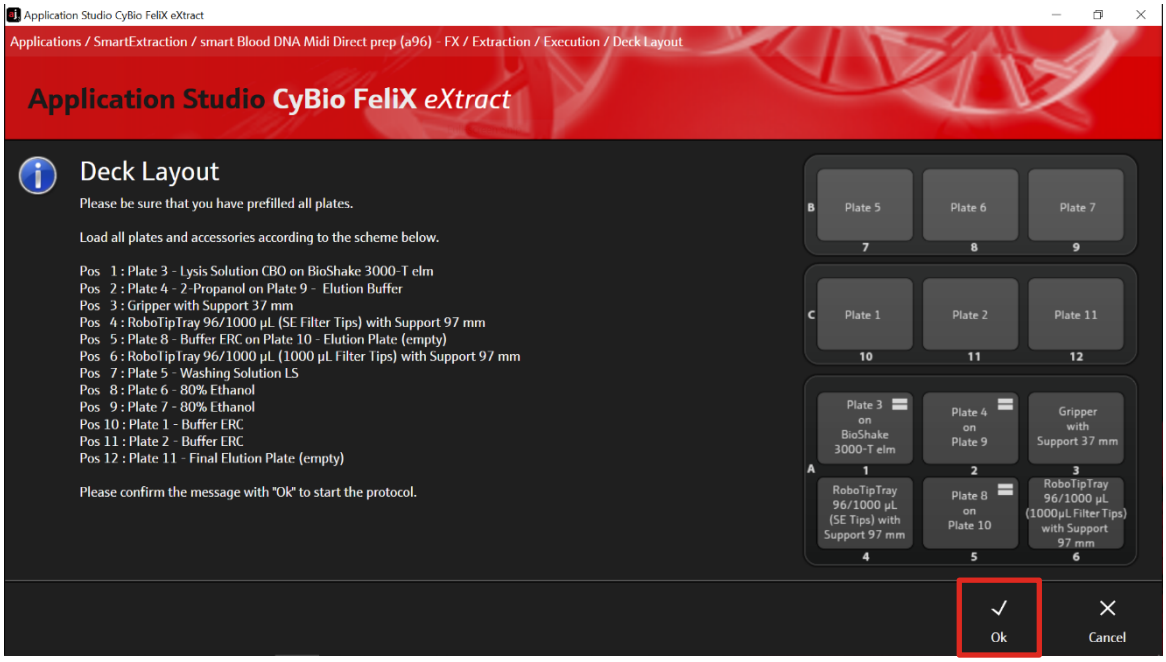


Figure 36: Deck layout for checking the correct positions of all plates and accessories.

10. The chosen extraction protocol is performed by the device. After the protocol is finished, the message “Purification process completed” is displayed. Confirm the message with “Ok” (→ see **Figure 37**).

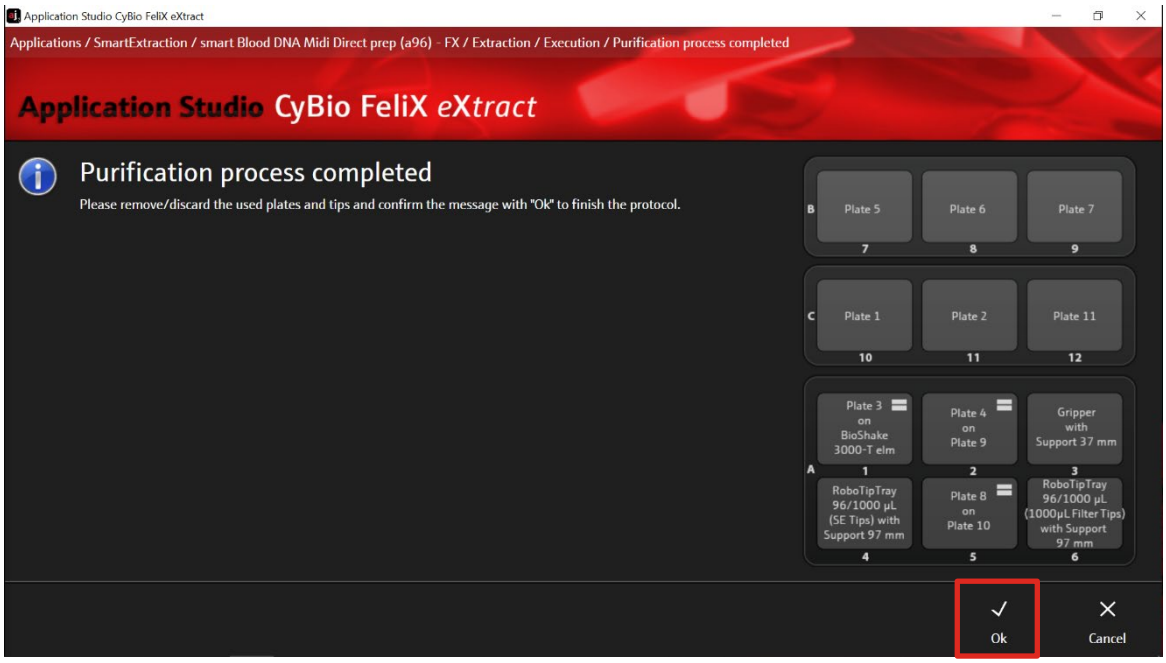


Figure 37: Purification process completed.

11. Once the extraction protocol is finished, remove **Plate 11 – Final Elution Plate** from **deck position 12** or **Plate 10 – Elution Plate** (→ see Note on p. 42) from the BioShake 3000-T-elm on **deck position 1**.
12. Seal the respective plate with the included sealing film and store DNA under adequate conditions.

NOTE

When using alternate elution vessels as listed in (→ see "Loading of CyBio FeliX", p. 40), proceed analogously.
Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C. For long time storage we recommend -80 °C.

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

15 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
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 page 23 or section 14.1 on page 40).
High viscosity extracted DNA	
Insufficient amount of Elution Buffer	Elute the DNA with a higher volume of Elution Buffer.
Degraded or sheared DNA	
Old sample material	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion.

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