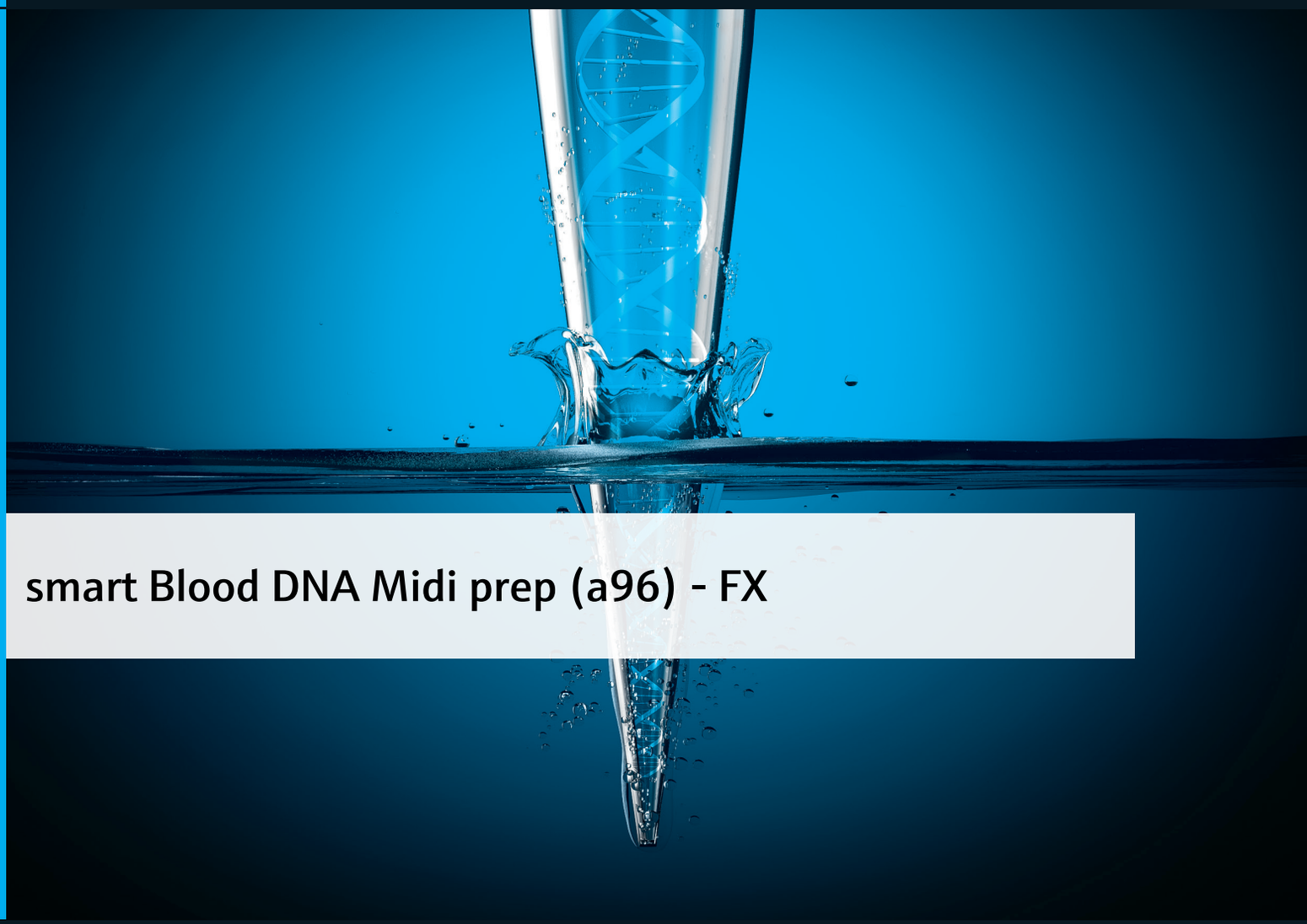
Instructions for Use
Life Science Kits & Assays



smart Blood DNA Midi prep (a96) - FX

iST Innuscreen GmbH

Order No.:
845-FX-4196096 96 reactions
845-FX-4196480 480 reactions

Publication No.: HB_FX-4196_e_220608

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 prep (a96) – FX** has been designed for automated isolation of high molecular weight genomic DNA (gDNA) from peripheral blood mononuclear cells (PBMCs) derived from fresh or frozen blood stabilized with EDTA, citrate, heparin and PAXgene® Blood DNA Tubes. The kit utilizes the new SmartExtraction technology.

The procedure starts with the lysis of erythrocytes and the subsequent pelleting of the PBMCs. After addition of PBS the cells are resuspended and transferred into a Reagent Plate of the kit.

The extraction process is based on adsorption of the 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 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.
15 °C 30 °C	**Storage conditions** Store at room temperature, unless otherwise specified.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. →"Notes on the use of this manual", p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before use to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information.

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. This kit is to be used with potentially infectious human samples. 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 should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

All kit components are shipped at ambient temperature.

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

All other components of the **smart Blood DNA Midi prep (a96) – FX** 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 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 was produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the smart Blood DNA Midi 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. 9). 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 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-4196096	845-FX-4196480
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
Ery Lysis Solution A (conc.)	100 mL	4 x 100 mL
Ery Lysis Solution B (conc.)	60 mL	3 x 100 mL
Lysis Solution CBO	50 mL	2 x 100 mL
Washing Solution LS (conc.)	15 mL	5 x 15 mL
Elution Buffer	70 mL	3 x 110 mL
Deep Well Plate (2.0 mL)	7	35
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

- 1.5 mL, 2.0 mL and 15 mL tubes
- ddH_2O for dissolving **Proteinase K**
- optional RNase A (10 mg/mL)
- 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, undenaturated)
- 2-Propanol (molecular biology grade)
- 96 % – 99.8 % Ethanol (molecular biology grade, undenaturated)
- 1 column and 3 column reservoirs for prefilling by CyBio FeliX (Smart Prefilling Set 1, 5x96 reactions, OL3317-25-128)

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, Analytik Jena GmbH)
- System-specific, pre-configured Laptop (820-90002-2, Analytik Jena GmbH)

6.4 Related products

- Protective Plate (31-01641, 10 pcs, IST Innucreen 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 Innucreen GmbH)
- Deep Well Plate (96 square well, 2.0 mL) (845-FX-8500115, 115 pcs, IST Innucreen 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

- 0.5–3 mL whole blood (fresh or frozen) stabilized with EDTA, citrate or heparin or sampled with PAXgene® Blood DNA Tubes.

2. Time for isolation

Lysis (external)	Automated prefilling	Extraction	Elution volume
approx. 20 min	21 min	45 min	150–500 µL

3. Typical yield

Whole blood volume	Typical yield
0.5 mL	5–15 µg
1.0 mL	15–40 µg
2.0 mL	40–70 µg
3.0 mL	50–90 µg

NOTE

Yield of isolated DNA is affected by amount and condition of PBMCs used. The condition of PBMCs depends on storage conditions as well as constitution of the donor. It has to be considered that a medical attendance of the donor may lower the yield of isolated DNA. This kit requires intact cells and may not work satisfying in case of damaged cells in starting material!

8 Initial steps before starting

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

845-FX-4196096 845-FX-4196480	Add 1.5 mL ddH_2O to lyophilized Proteinase K.

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

845-FX-4196096 845-FX-4196480	Add 60 mL ethanol to 15 mL Washing Solution LS (conc.).

- Mix **100 mL** of **Ery Lysis Solution A (conc.)** with **900 mL ddH_2O** in an appropriate bottle. Always keep the bottle firmly closed!
- Mix **Ery Lysis Solution B (conc.)** with the indicated amount of ddH_2O in an appropriate bottle. Always keep the bottle firmly closed!

845-FX-4196096	Add 540 mL dd H_2O to 60 mL Ery Lysis Solution B (conc.).
845-FX-4196480	Add 900 mL dd H_2O to 100 mL Ery Lysis Solution B (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

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

NOTE
Prefilling of Deep Well Plates can be carried out during lysis of samples. Ensure that the **Washing Solution LS** has been prepared according to the instructions (→ see "Initial steps before starting", p. 10).

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**).

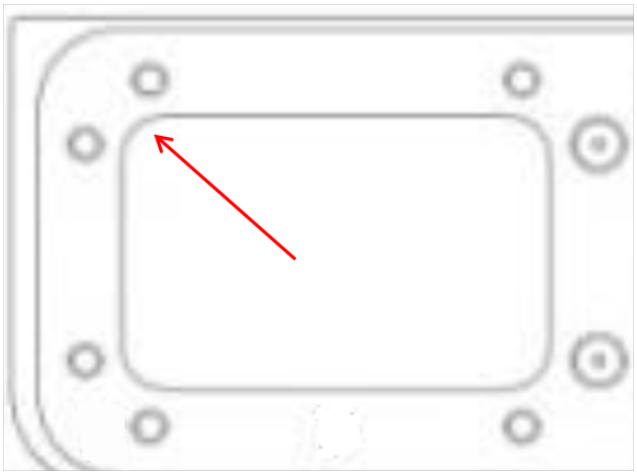

Figure 2: Positioning of plates and reservoirs on CyBio FeliX deck.

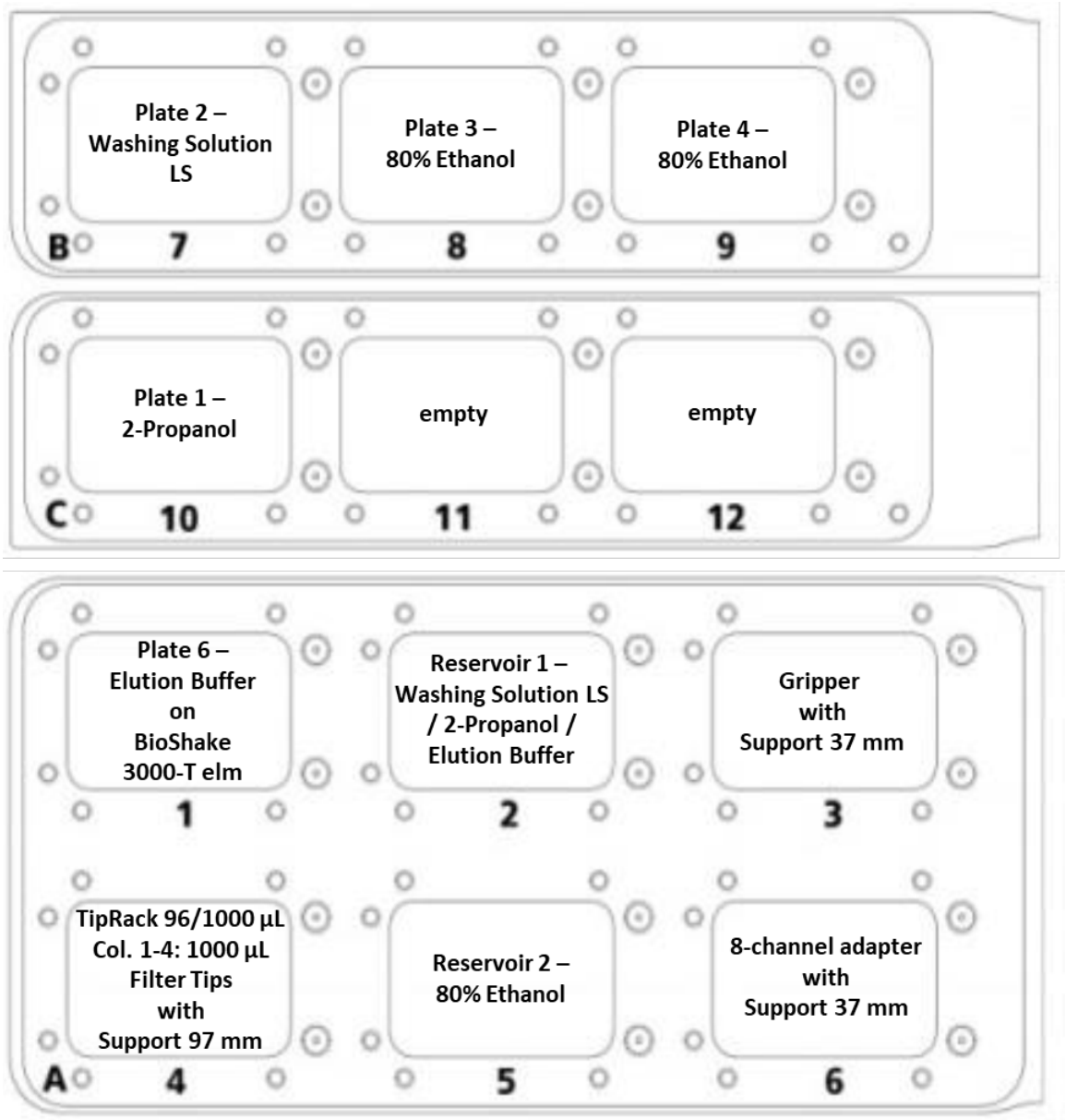

Figure 3: Deck Layout for the prefilling protocol.

NOTE

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

1. Label the 3 column reservoir and the 1 column reservoir from the Smart Prefilling Set 1(→ see section 6.2 on p.7) according to the table below:

Number	Label	
Reservoir 1 (3 column)	**Reservoir 1:** Left side of reservoir: Middle position: Right side of reservoir:	 Washing Solution LS 2-Propanol 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 Plate (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 **Elution Buffer** (70 mL) 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 Tip Rack 96/1000 µL. Please fill the whole rows of the columns with filter tips.
8. Place the 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 the deck layout for the prefilling protocol (→ see **Figure 3**).

NOTE
Please pay special attention to the following deck position:

Position 1:
Place **Plate 6 – Elution Buffer** directly on the BioShake 3000-T elm.

11. Switch on the CyBio FeliX and open AppStudio FeliX *eXtract*.
12. Select the extraction technology "SmartExtraction" (→ see **Figure 4**) and the extraction kit "smart Blood DNA Midi prep (a96) – FX" (→ see **Figure 5**).

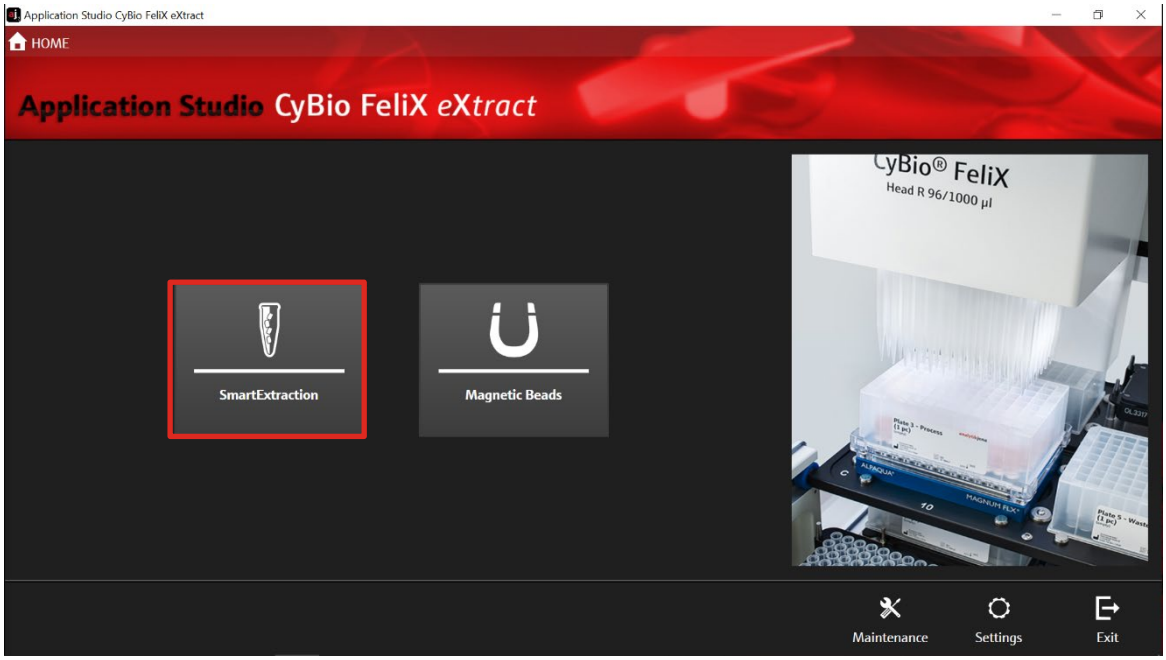

Figure 4: HomeScreen of the AppStudio FeliX eXtract. Selection of extraction technology: “SmartExtraction”.

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

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

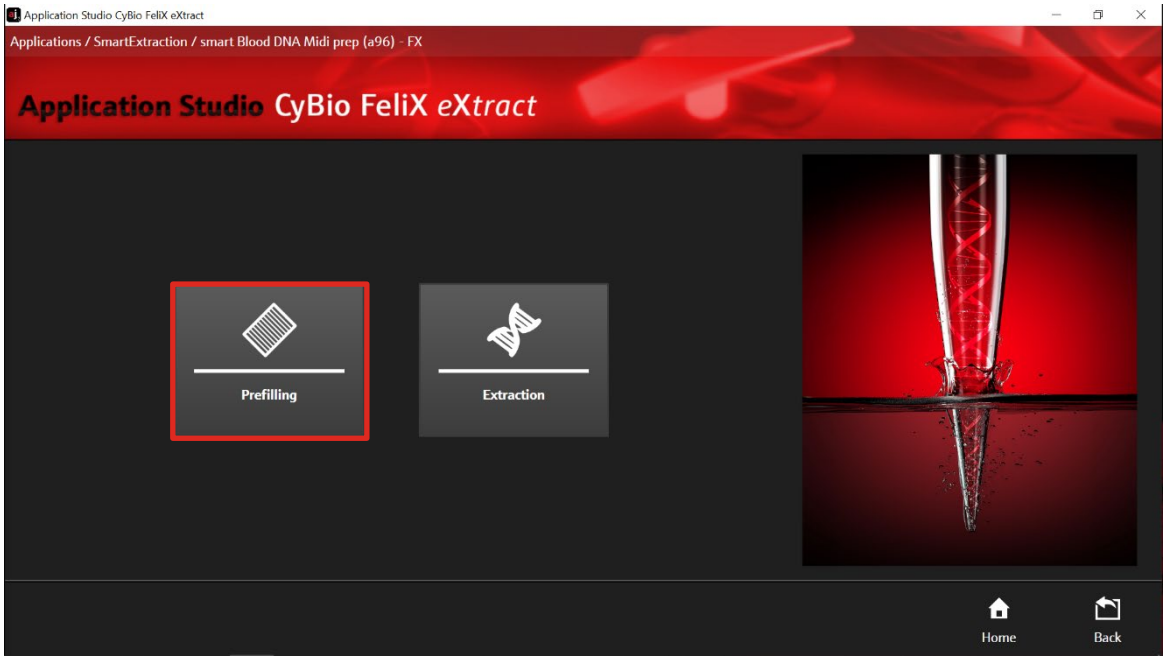

Figure 6: Routine selection: Prefilling.

15. After selecting “Prefilling” the Prefilling Start Screen appears.

16. Check the correct version number of the protocol: “Prefilling – SE External Lysis (a96) – 01” and confirm with “Execute” (→ see **Figure 7**).

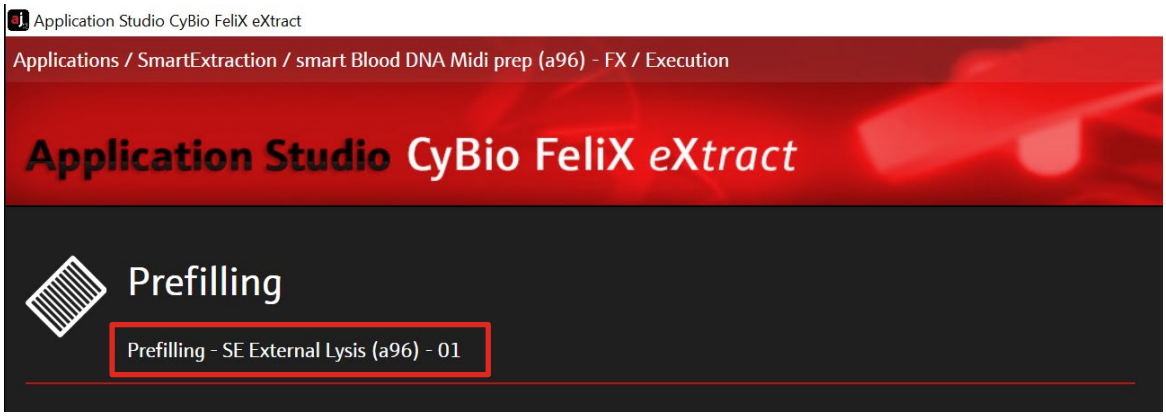

Figure 7: Version number of the prefilling protocol.

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

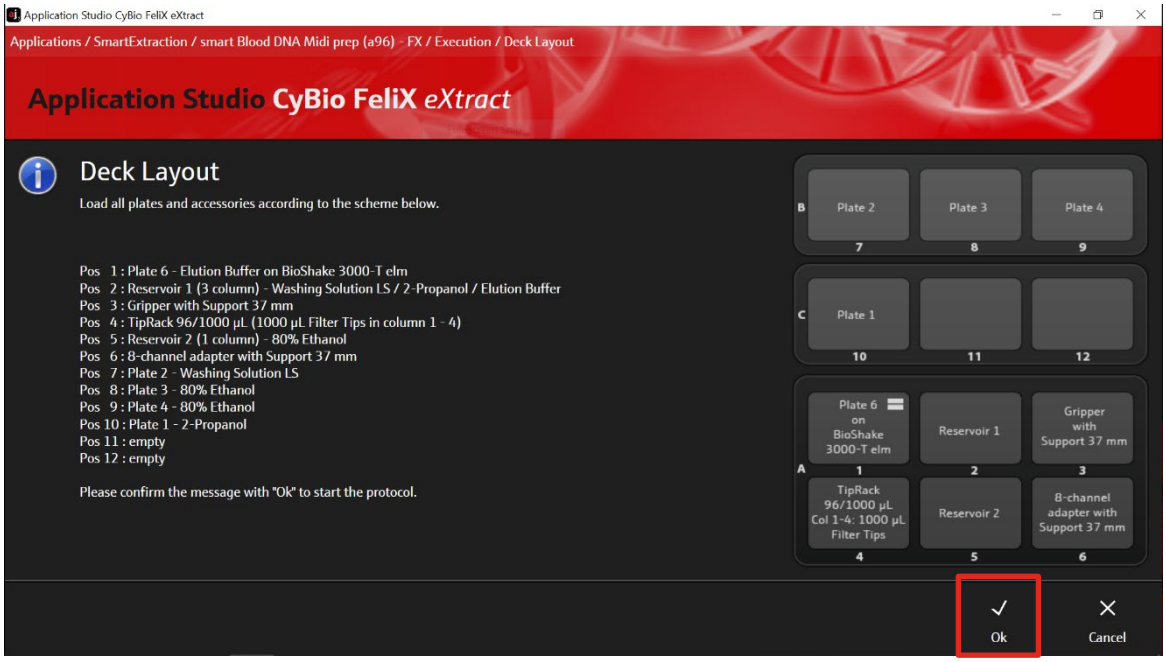

Figure 8: Deck layout for checking the right positions of all plates and accessories.

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: 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.

9.2 Manual prefilling of Reagent Plates

NOTE
Prefilling of Deep Well Plates can be carried out during lysis of samples. Ensure that the **Washing Solution LS** has been prepared according to the instructions (→ see "Initial steps before starting", p. 10).

Label and fill the required wells 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

NOTE
The deep well plates do not have to be filled completely. If less than 96 samples are to be extracted, only the required wells must be prefilled.
The lysed sample will be processed using CyBio FeliX as a liquid handling platform. Please p**ay special attention to section "**Loading the sample and starting CyBio FeliX**"** on page 23**.**

10 Preparation of blood samples

NOTE

The kit has been optimized for isolation of genomic DNA from PBMCs derived from 0.5–3.0 mL fresh or frozen whole blood.

10.1 Sample preparation of 1-3 mL whole blood

1. Dispense **Ery Lysis Solution A** according to the volume of whole blood sample (see table below) into a 15 mL tube.

Whole blood volume	Volume of Ery Lysis Solution A
1.0 mL	3.0 mL
2.0 mL	5.0 mL
3.0 mL	8.0 mL

2. Add **1.0 mL**, **2.0 mL** or **3.0 mL whole blood** into the prepared 15 mL tube and mix by inverting 6 times.
3. Incubate 5–10 min at room temperature. Invert at least once during incubation time.

NOTE

For fresh blood (collected within 1–6 h before starting the extraction) increase incubation time to 20 min to ensure complete lysis of red blood cells.

4. Centrifuge for 5 min at 2,500 x g to pellet the PBMCs.
5. Carefully discard the supernatant by pipetting or pouring.

NOTE

Do not discard the PBMC pellet.

6. Add **5 mL Ery Lysis Solution B** to the PBMC pellet and vortex briefly.
7. Centrifuge for 5 min at 2,500 x g to pellet the PBMCs.
8. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet. Use a paper towel to remove residual liquid as much as possible!

9. Add **120 µL PBS** to the PBMC pellet and resuspend the cells as much as possible by intensive pipetting up and down.
10. Proceed with section 10.3 “Sample Lysis” on page 22.

10.2 Sample preparation of 0.5 mL whole blood

1. Add **0.5 mL whole blood** into a 2.0 mL tube.
2. Add **1.0 mL of Ery Lysis Solution A** to the tube and vortex for 5 seconds.
3. Incubate 5–10 min at room temperature. Invert at least once during the incubation time.

NOTE
For fresh blood (collected within 1–6 h before starting the extraction) increase incubation time to 20 min to ensure complete lysis of red blood cells.

4. Centrifuge for 5 min at 2,500 x g to pellet the PBMCs.
5. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet.

6. Add **1 mL Ery Lysis Solution B** to the PBMC pellet and vortex briefly.
7. Centrifuge for 5 min at 2,500 x g to pellet the PBMCs.
8. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet. Use a paper towel to remove residual liquid as much as possible!

9. Add **200 µL PBS** to the PBMC pellet and resuspend the pellet as much as possible by intensive pipetting up and down.

10. Proceed with section 10.3 “Sample Lysis” below.

10.3 Sample Lysis

1. Add **200 µL Lysis Solution CBO** and **Proteinase K** according to the table below to the resuspended PBMC pellet, mix vigorously by pulsed vortexing for 10 sec.

Whole blood used	Proteinase K to be added
0.5 mL	40 µL
1.0 mL	40 µL
2.0 mL	40 µL
3.0 mL	50 µL

2. Incubate at 50 °C until the pellet is completely lysed (about 25 min).

3. Proceed with section 11.2 “Loading the sample and starting CyBio FeliX” on page 23.

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**.

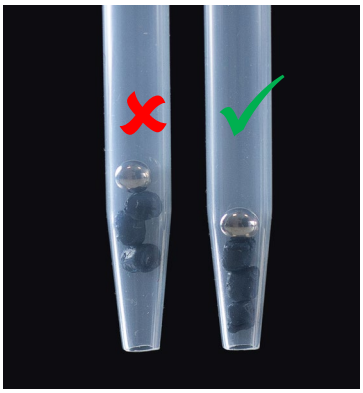

Figure 10: Checking SmartExtraction Tips.

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 10.

11.2 Loading the sample and starting CyBio FeliX

1. Transfer the lysate (max 400 µL) into **Plate 1 – 2-Propanol.**
2. Put **Plate 1 – 2-Propanol** on **deck position 10.**
3. Load all prepared plates and accessories onto 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 (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**.

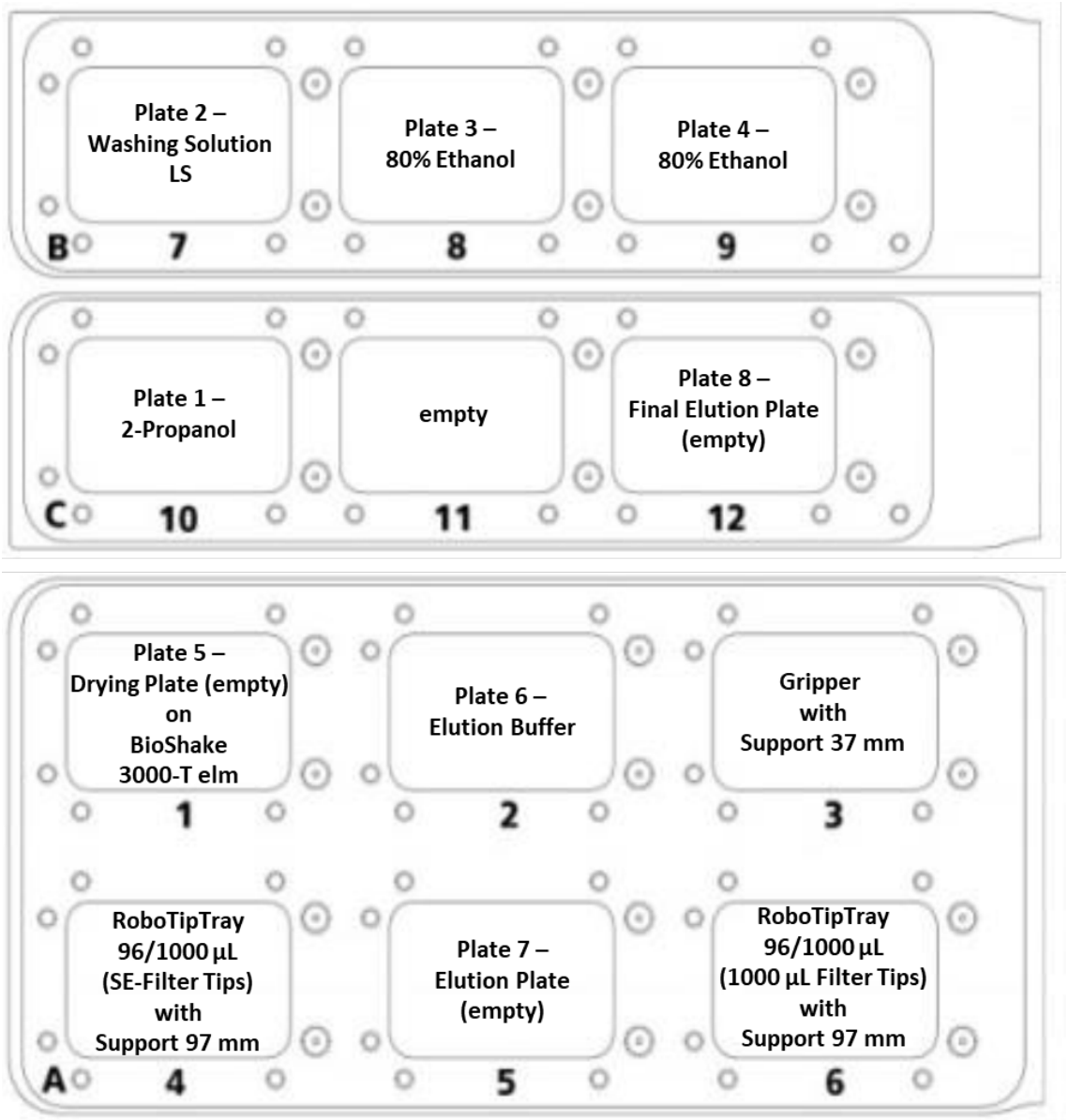

Figure 11: Deck Layout for starting the extraction protocol.

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 has to 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 CyBio FeliX *eXtract*.
5. Choose "SmartExtraction" (→ see **Figure 12**) and the kit "smart Blood DNA Midi prep (a96) – FX" (→ see **Figure 13**).

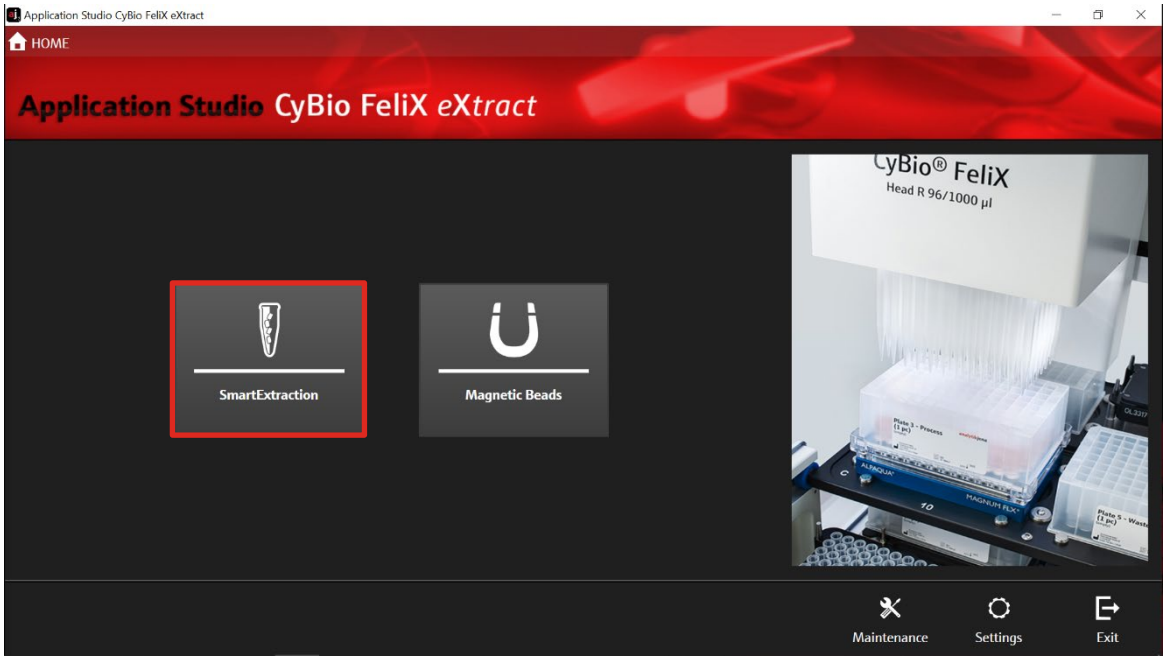

Figure 12: Homescreen AppStudio FeliX eXtract. Selection of extraction technology: SmartExtraction.

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

6. Check the correct protocol version “External Lysis (a96) – 02” (→ see **Figure 14**) and adjust the elution volume between **150-500 µL**. Recommended elution volumes are listed in the table below. Start the protocol by clicking the button “Execute” (→ see **Figure 15**).

Volume of whole blood sample	Recommended elution volume
0.5 or 1.0 mL	min. 200 µL
2.0 mL	300–400 µL
3.0 mL	300–500 µL

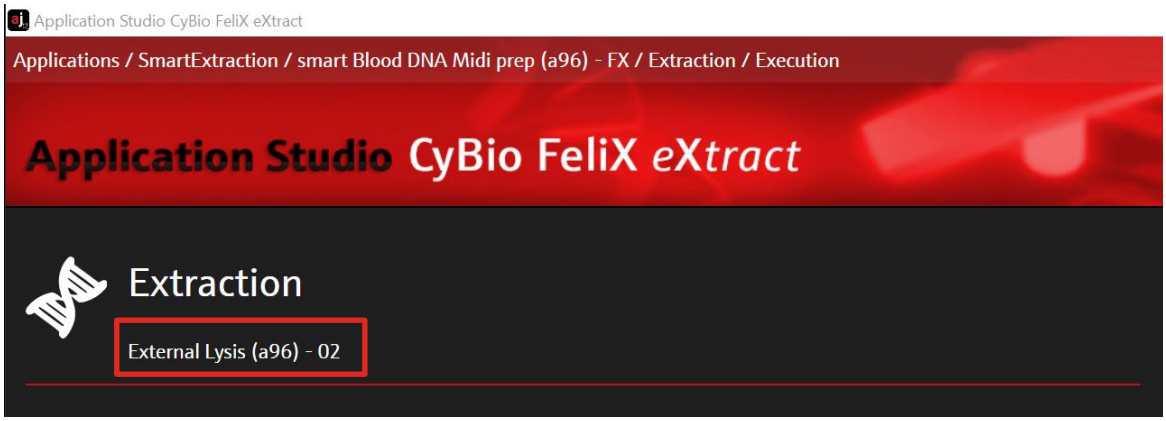

Figure 14: Version number of the extraction protocol.

Figure 15: Selection the elution volume (150 – 500 µL).

7. Check the correct positioning of plates on the corresponding deck positions and confirm with "Ok" (→ see **Figure 16**).

Figure 16: List deck positions and corresponding plates.

3. The chosen protocol is performed by the device. When the protocol is finished, the message "**Purification process completed**" is displayed. Confirm the message with "Ok" (→ see **Figure 17**).

Figure 17: Completion of extraction process.

4. Once the extraction is completed, remove **Plate 8 – Final Elution Plate** from **deck position 12** or **Plate 7 – Elution Plate** (→ see Note on p. 24) from the BioShake 3000-T-elm at **deck position 1**.
5. 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 the sample and starting CyBio FeliX”, p. 23, 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.

6. Afterwards, remove and discard the used Deep Well Plates and 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. Vigorously resuspend PBMC pellet.
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 22).
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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