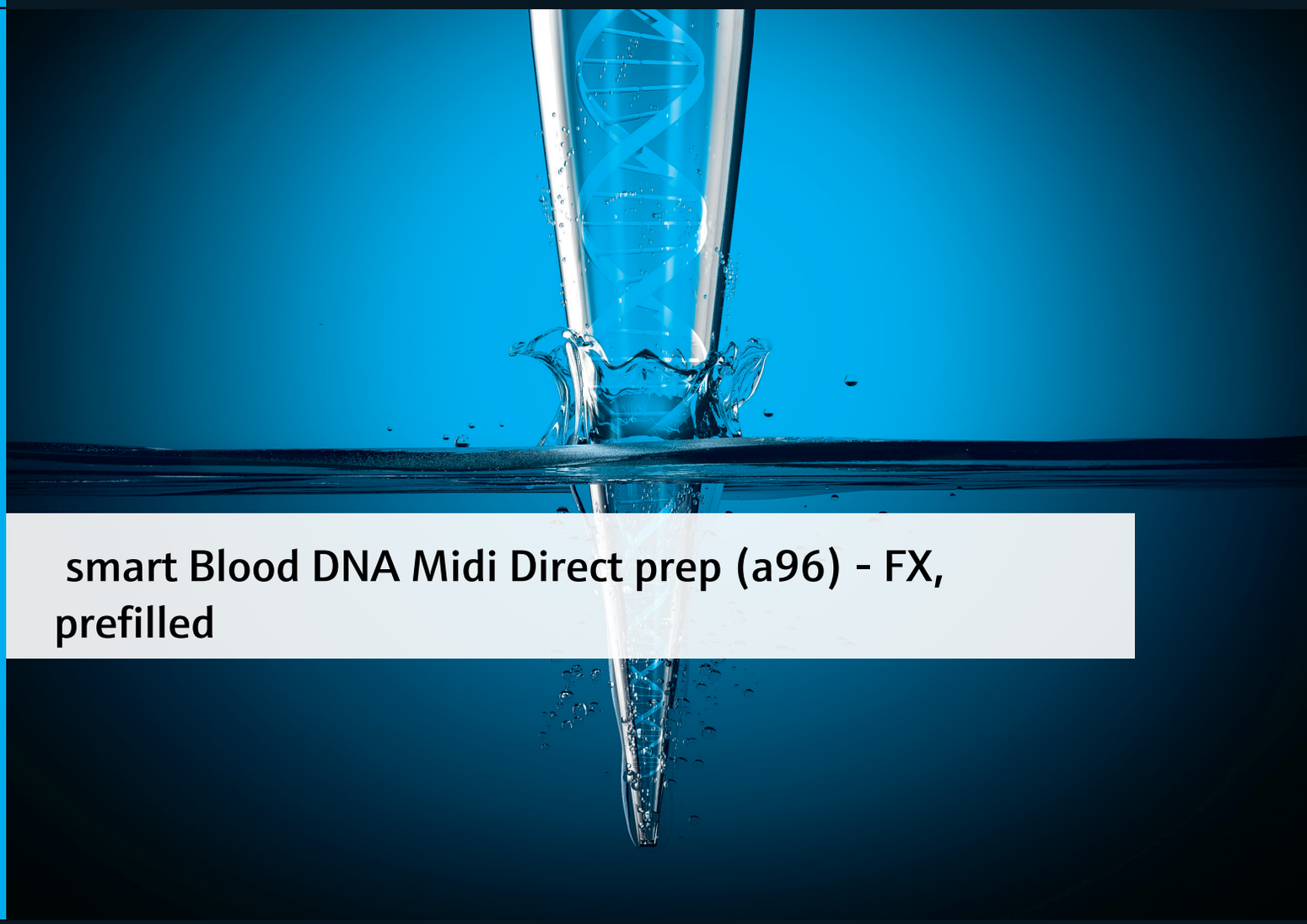
Instructions for Use

Life Science Kits & Assays



smart Blood DNA Midi Direct prep (a96) - FX, prefilled

iST Innuscreen GmbH

Order No.:
845-PFX-4296096 96 reactions

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

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Contents

1 Introduction

1.1 Intended use

The **smart Blood DNA Midi Direct prep (a96) – FX, prefilled kit** has been designed for automated isolation 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. The plates are already prefilled with the extraction reagents needed for the extraction.

The extraction process is based on adsorption of 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 required buffers are prefilled in 96 deep well plates. Therefore, hands-on-time for preparation is reduced to a minimum.

The whole extraction process simply requires pipetting up and down. The combination of patented low-salt DC-Technology with Smart Modified Surfaces 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. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before 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 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, 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.

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 Direct prep (a96) – FX, pre-filled” or other IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information please contact info.innu@ist-ag.com or your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications”, p. 8). 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
REF	845-PFX-4296096
SmartExtraction Tips	6 x 16
Proteinase K	for 4 x 1.5 mL working solution
Plate 1 – Buffer ERC (1050 µl)	1
Plate 2 – Buffer ERC (1050 µl)	1
Plate 3 – Lysis Solution CBO (400 µl)	1
Plate 4 – 2-Propanol (350 µl)	1
Plate 5 – Washing Solution LS (600 µl)	1
Plate 6 – 80 % Ethanol (600 µl)	1
Plate 7 – 80 % Ethanol (600 µl)	1
Plate 8 – Buffer ERC (1050 µl)	1
Plate 9 – Elution Buffer (600 µl)	1
Plate 10 – Elution Plate (empty)	1
Plate 11 – Final Elution Plate (empty)	1
Sealing Foil	1
Protective Plate	2
Filter Tips	96
Manual	1

6.2 Components not included in the kit

- ddH_2O for dissolving Proteinase K
- 1.5 mL and 2.0 mL tubes
- RNase A (10 mg/mL). optional

6.3 Required CyBio FeliX components

- CyBio FeliX Basic Unit with Enclosure and CyBio Composer Software (OL5015-24-100, Analytik Jena GmbH)
- CyBio FeliX Extraction Set (OL5015-25-120) including Application Studio 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:

- 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 maximum storage time of 24 hours at room temperature followed by a 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 material stabilized with **Citrate-Phosphate-Derivative (CPD) is not suitable** with this kit.

2. Time for isolation:

External lysis steps are not required.

Sample volume	Extraction time	Elution volume
0.5 mL	83 min	150-500 µL
1.0 mL	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 has to 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 starting material!

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! Usage of other supports or no supports may cause damage to the CyBio FeliX.

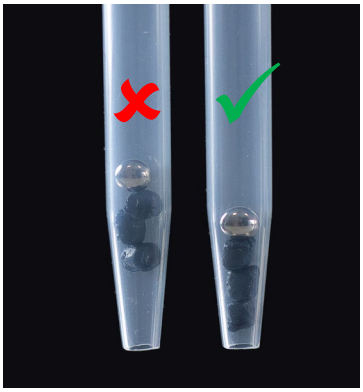
See Figure 1 in order to differentiate between CyBio RoboTipTray 1-96/1000 µL and CyBio TipRack 96/1000 µL.

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

9 Preparatory steps for automated extraction

9.1 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 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 Smart Extraction Tips

9.2 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 min 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 interphase and transfer into a new 1.5 mL tube.

9.3 Preparation of Reagent Plates

9.3.1 Unpacking Reagent Plates

NOTE
According to transport regulations Reagent Reservoirs 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.

9.3.2 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!

10 Loading the sample and starting CyBio FeliX

The **smart Blood DNA Midi Direct prep (a96) – FX, prefilled** kit is optimized for sample volumes ranging from 200–1000 µL of whole blood. Due to the wide volume range, preparation differs for smaller and larger sample volumes (→ see sections 10.1 and 9.6.2, respectively).

10.1 Using 200–500 µL sample material

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**. Please pay attention to transfer the blood sample or buffy coat first 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	-

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 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 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**.

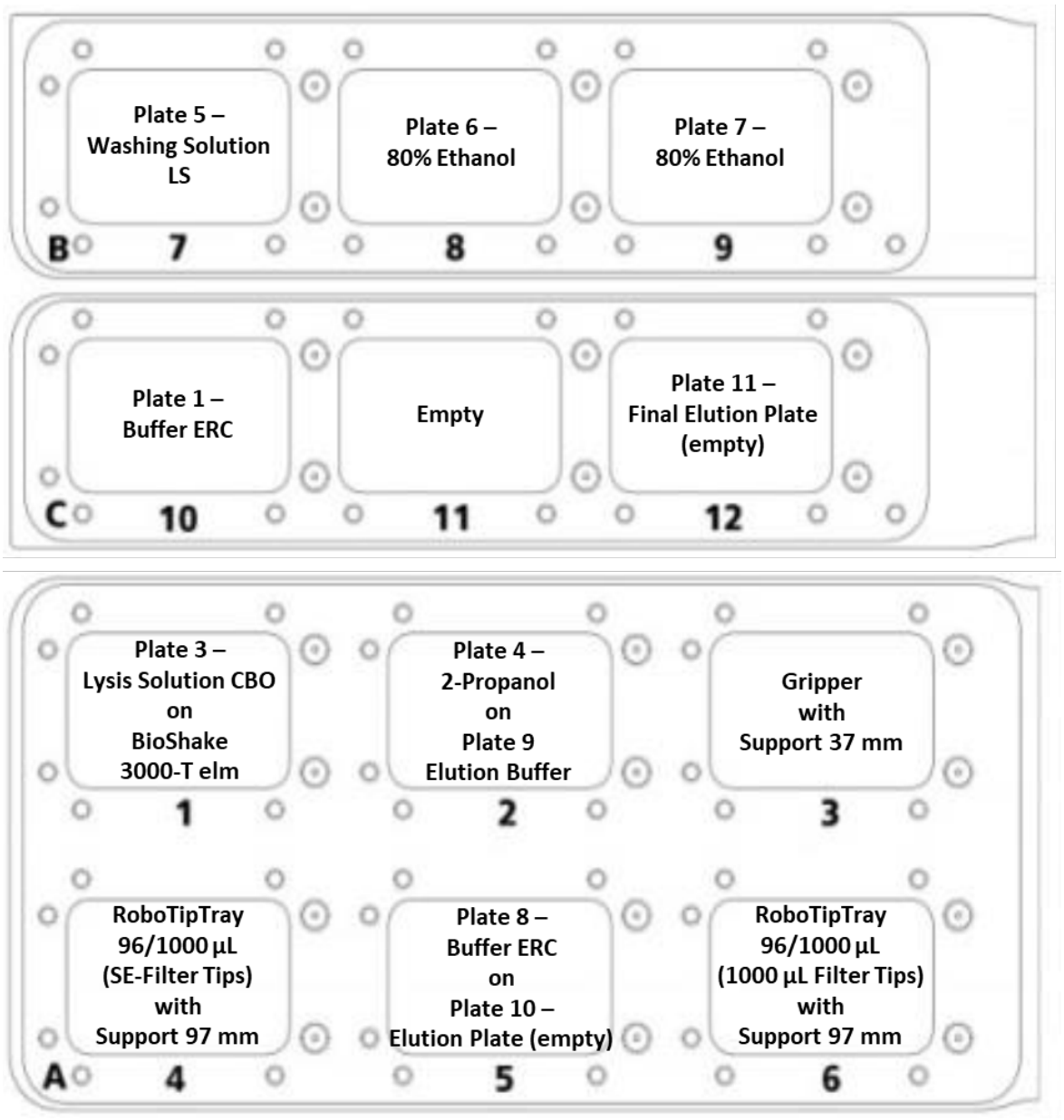


Figure 3: Deck layout for the extraction of 200–500 µL sample material.

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 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. Choose “SmartExtraction” and the kit “smart Blood DNA Midi Direct prep (a96) – FX, prefilled” (→ see **Figure 4** and **Figure 5**).

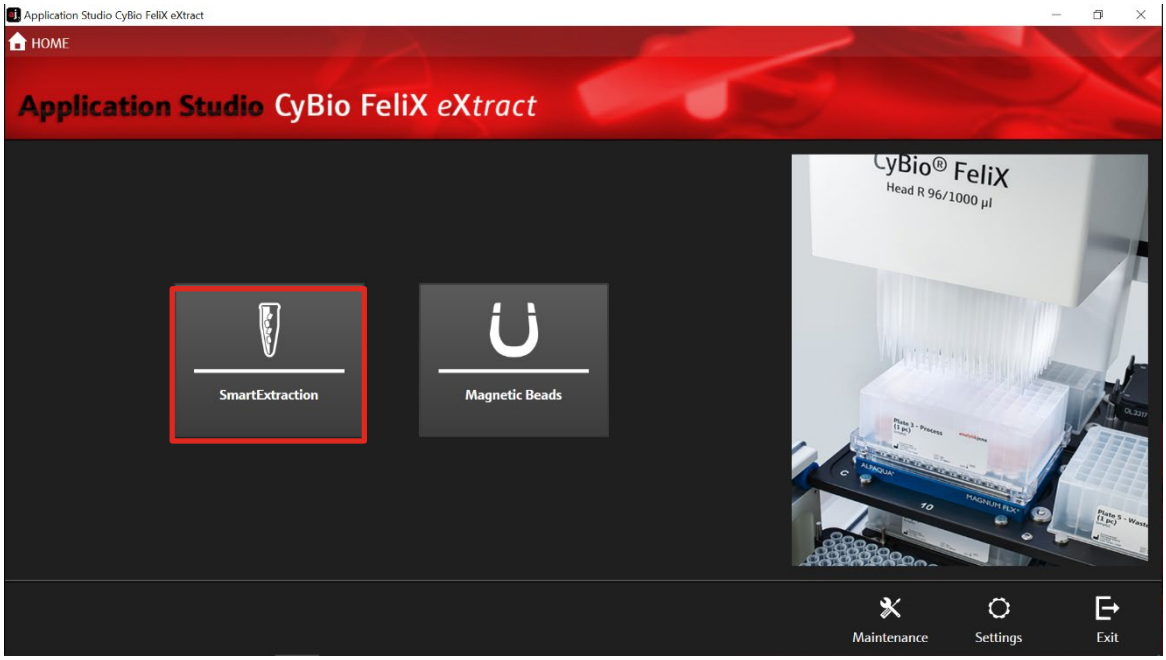


Figure 4: Homescreen AppStudio FeliX *eXtract*. Selection of extraction technology: SmartExtraction.

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

6. Check the correct protocol version “Internal Lysis (a96) – 02” (→ see **Figure 6**).

Figure 6: Version number of the extraction protocol.

7. Select the protocol for up to 500 µL sample volume and adjust the elution volume between **150-500 µL.** Start the protocol by clicking “Execute” (→ see **Figure 7**).

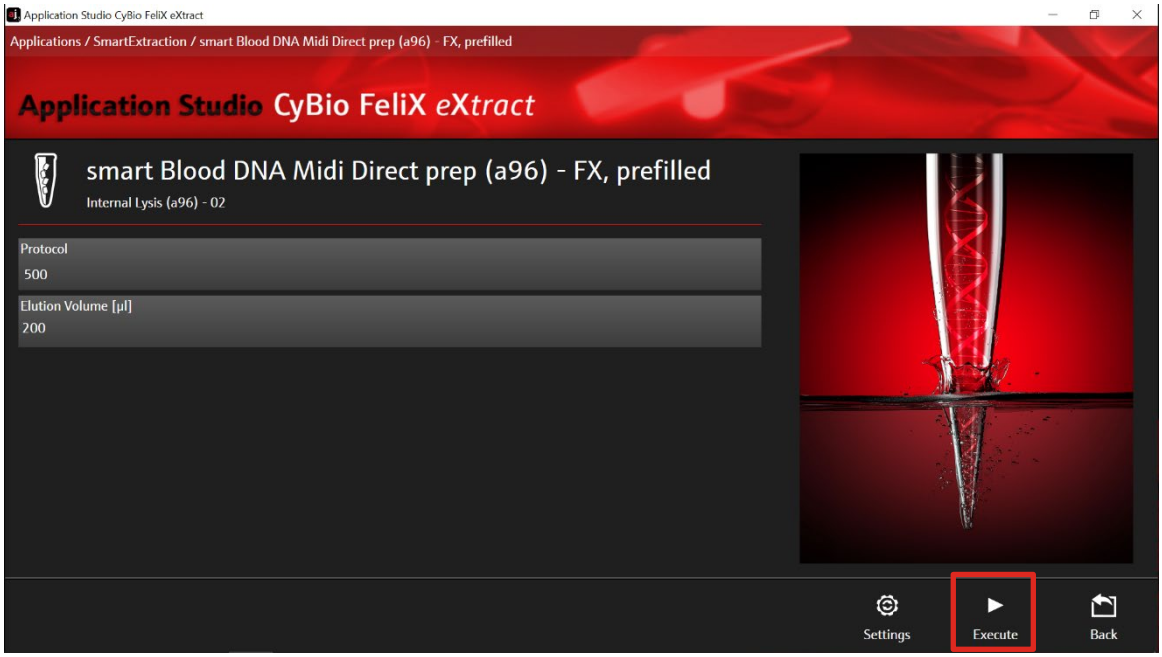


Figure 7: Selection of sample volume (500 µL) and elution volume (variable).

8. Check the correct positioning of plates on the corresponding deck positions (→ see **Figure 8**) and confirm with "Ok".

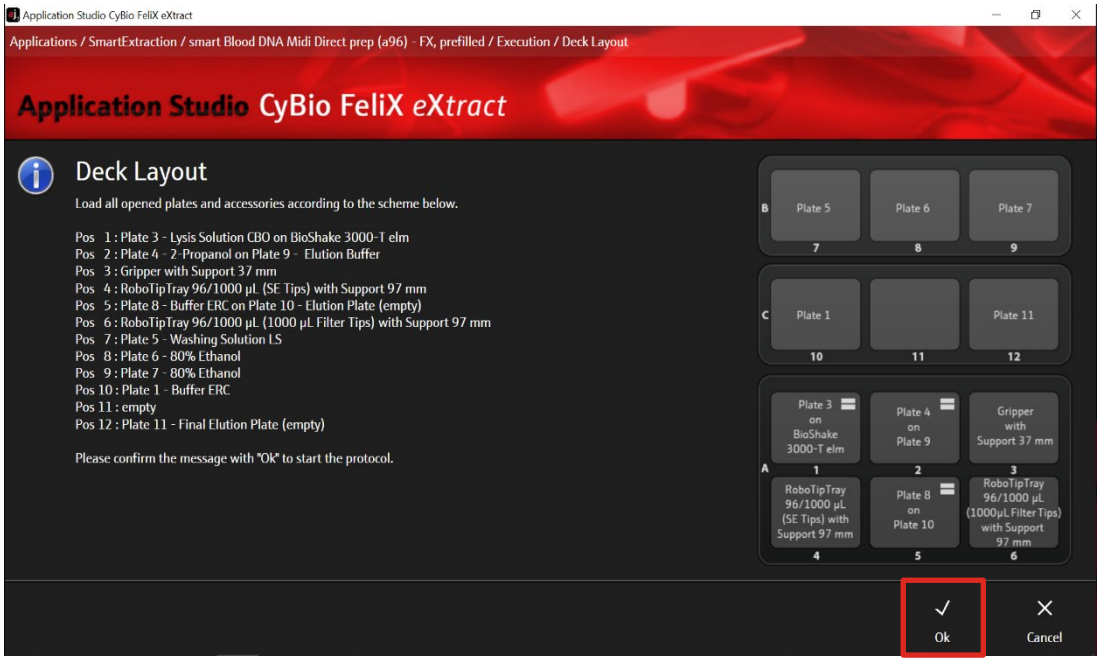


Figure 8: List of deck positions and corresponding plates.

9. 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 9**).

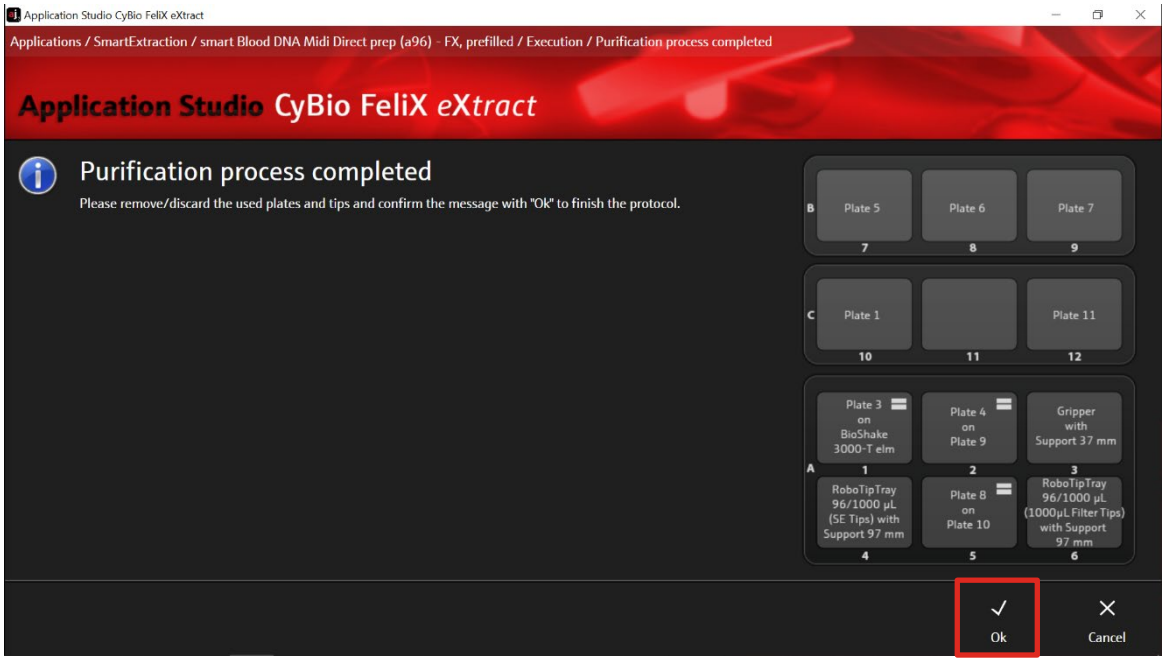


Figure 9: Process completed.

10. Once the extraction is completed, remove **Plate 11 – Final Elution Plate** from **deck position 12** or **Plate 10 – Elution Plate** (→ see Note on p. 15) from the BioShake 3000-T-elm on **deck position 1**.
11. 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” on page 12, proceed analogously.
Store the DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C. For long time storage placing at -80 °C is recommended!

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

10.2 Using 600–1000 µL sample material

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** and **Plate 2 – Buffer ERC**. Please pay attention to transfer the blood sample or buffy coat first and then transfer the water.

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

3. Load all plates and accessories onto CyBio FeliX decks according to **Figure 10Fehler! Verweisquelle konnte nicht gefunden werden.** 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 plate of the **97 mm support**!

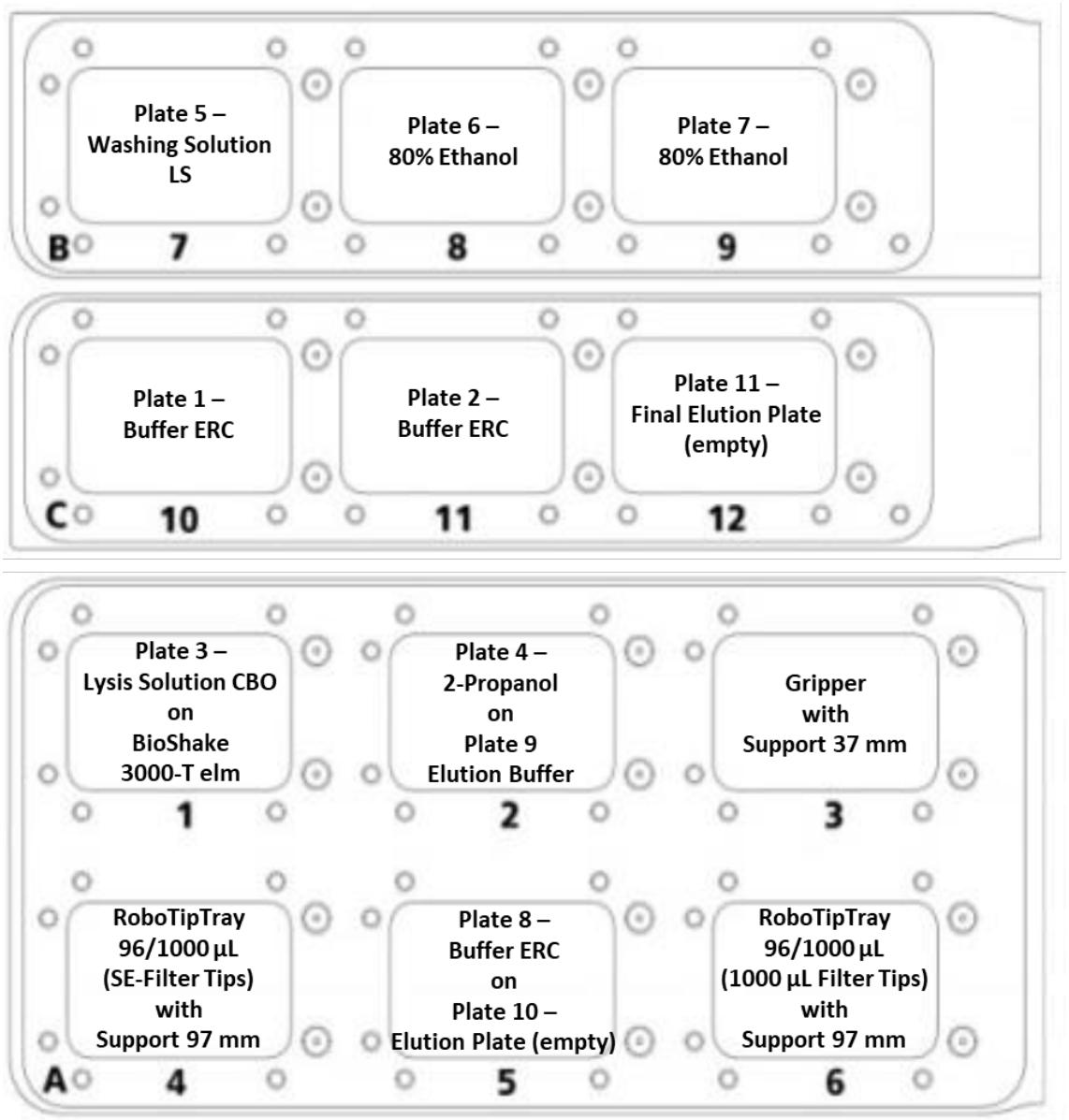


Figure 10: Deck layout for the extraction of 600–1000 µL sample material.

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 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 (e.g. Elution Tubes with Elution Caps or 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 11**).

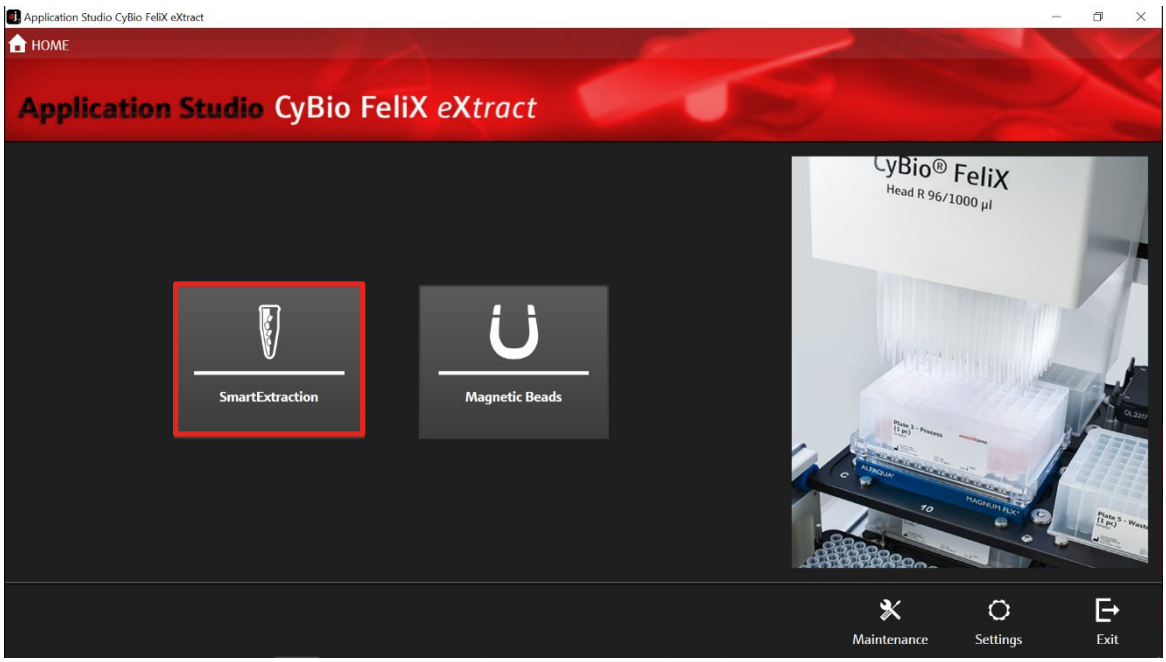


Figure 11: Homescreen of AppStudio FeliX eXtract. Selection of extraction technology: SmartExtraction.

6. Select the extraction kit "smart Blood DNA Midi Direct prep (a96) – FX, prefilled" (→ see **Figure 12**).

Figure 12: Kit selection: smart Blood DNA Midi Direct prep (a96) – FX, prefilled.

7. Check the correct protocol version: “Internal Lysis (a96) – 02” (→ see **Figure 13**)

Figure 13: Version number of extraction protocol.

8. Select the protocol for 1000 µL sample volume and adjust the elution volume between **150-500 µL.** Start the protocol by clicking “Execute” (→ see **Figure 14**).

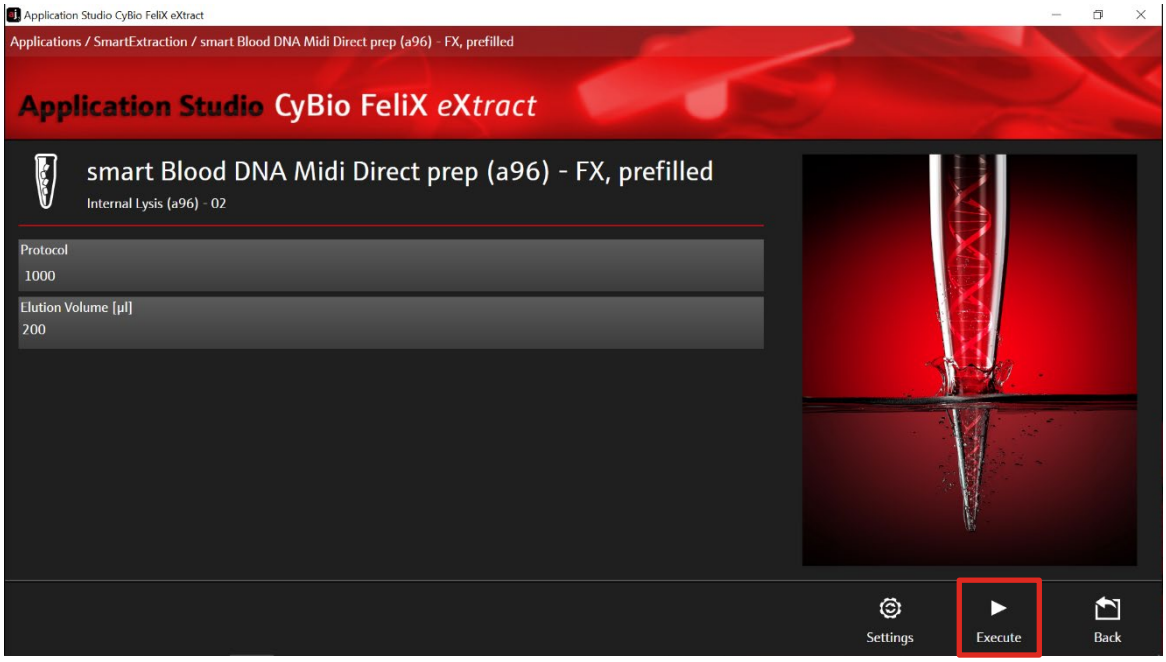


Figure 14: Selection of sample volume (1000 µL) and elution volume (variable).

9. Check the correct positioning of plates and accessories on the corresponding deck positions (→see **Figure 15**) and confirm with “Ok”.

Figure 15: Deck layout for checking the correct positions of all plates and accessories.

10. The chosen protocol is performed by the device and after the protocol is finished the message “**Purification process completed**” is displayed. Confirm the message with “Ok” (→ see **Figure 16**).

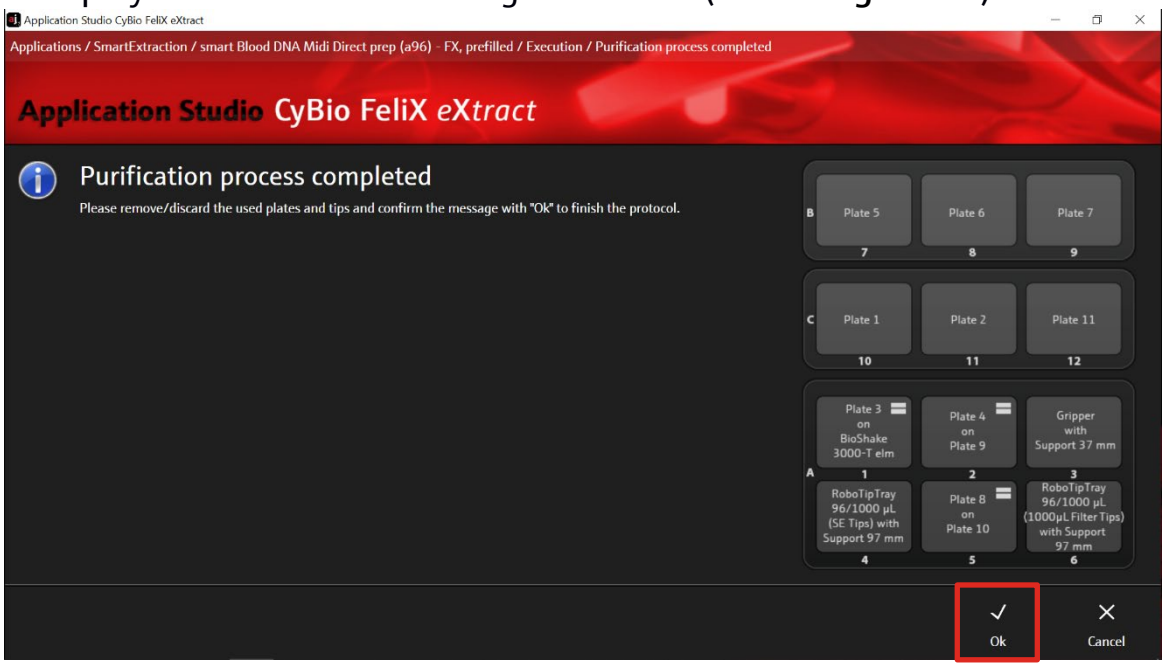


Figure 16: Purification process completed.

11. Once the extraction protocol is completed remove **Plate 11 – Final Elution Plate** from **deck position 12** or **Plate 10 – Elution Plate** (→ see Note on p. 20) 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 “Loading the sample and starting CyBio FeliX” on page 12, proceed analogously.
> Store the DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C. For long time storage placing at -80 °C is recommended!

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

11 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 9.1 on page 11).
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 insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

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