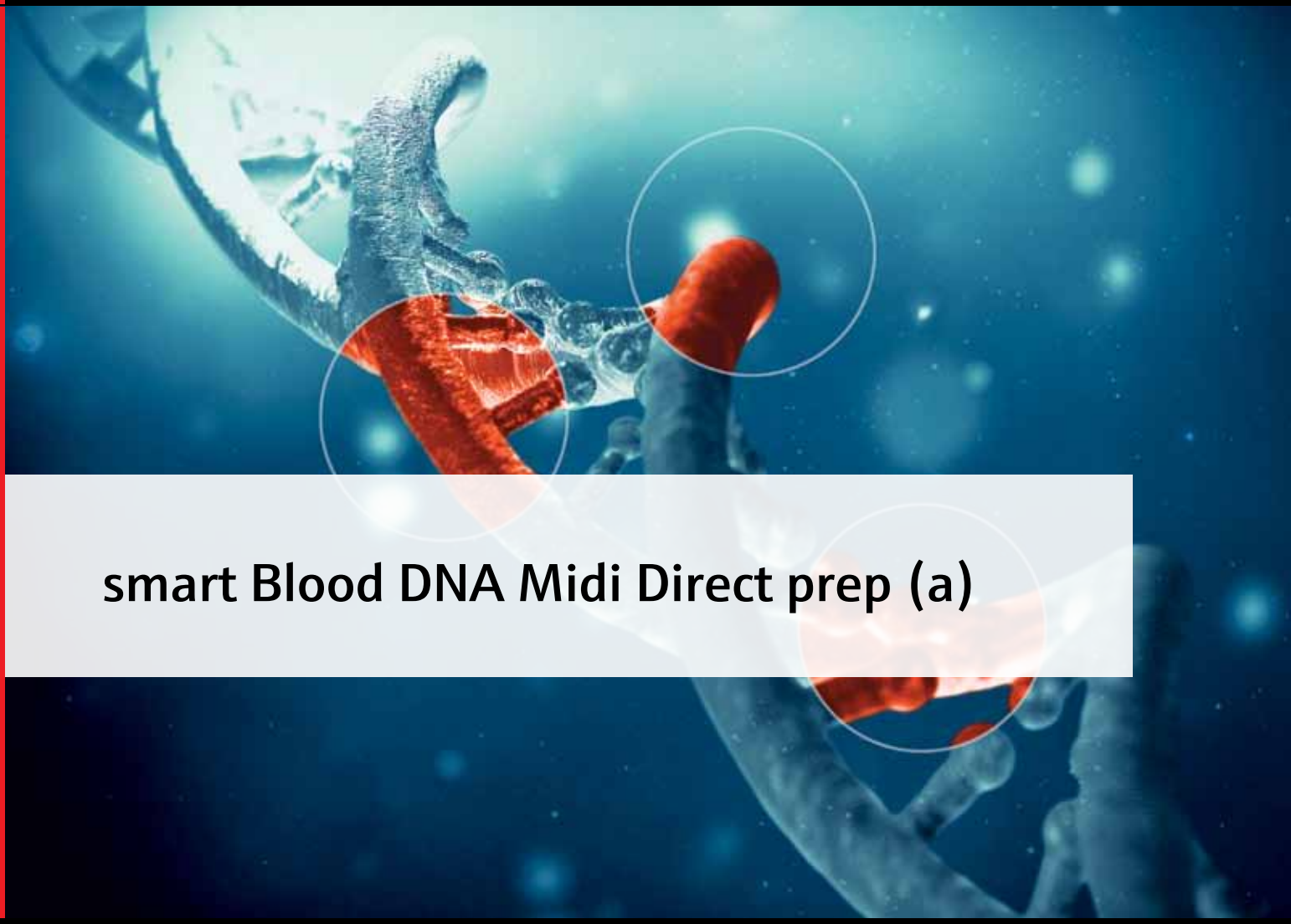
Instructions for Use
Life Science Kits & Assays



smart Blood DNA Midi Direct prep (a)

analytikjena
An Endress+Hauser Company

Order No.:
845-ASS-3008016 16 reactions
845-ASS-3008096 96 reactions
845-ASP-3008016 16 reactions
845-ASP-3008096 96 reactions

Publication No.: HB_ASP-3008_e_180212

Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin
Made in Germany!

Distribution/Publisher:
Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena · Germany

Phone +49 3641 77 9400
Fax +49 3641 77 767776
www.analytik-jena.com
info@analytik-jena.com

Contents

1 Introduction 3
1.1 Intended use 3
1.2 Notes on the use of this manual 4
2 Safety precautions 5
3 Storage conditions 6
4 Function testing and technical assistance 7
5 Product use and warranty 7
6 Kit components 8
7 GHS Classification 10
7.1 Hazard phrases 11
7.2 Precaution phrases 12
8 Product specifications 13
9 Preparation of buffy coat from whole blood 14
10 Preparation of Reagent Plates or Reagent Strips 15
10.1 General filling scheme 15
10.2 Unpacking of Reagent Plates or Strips and piercing of sealing foil 16
11 Automated extraction using InnuPure® C16/C16 *touch* 19
11.1 Sample tray of InnuPure® C16/C16 *touch* 19
11.2 Preparing sample tray of InnuPure® C16 / C16 *touch* 20
11.3 Handling of SmartExtraction Pipette Tips 22
11.4 Loading the sample to InnuPure® C16/C16 *touch* 23
11.5 Starting the InnuPure® C16 23
11.6 Starting the InnuPure® C16 *touch* 26
12 Automated extraction using CyBio® FeliX 29
12.1 Accessories needed 29
12.2 Preparing CyBio® FeliX 30
12.3 Handling of SmartExtraction Tips 32

12.4 Loading the sample and starting CyBio® FeliX 32
13 Troubleshooting 35
14 Related Products 36

1 Introduction

1.1 Intended use

The smart Blood DNA Midi Direct prep (a) has been designed for automated isolation of high molecular weight genomic DNA from whole blood samples stabilized with EDTA, citrate or heparin. The kit utilizes the new SmartExtraction technology using Smart Modified Surfaces invented by Analytik Jena AG (patent pending).

The sample is transferred into Reagent Strips or Reagent Plate of the kits, which are already prefilled with all extraction reagents needed for the automated isolation process using a unique 1 ml filter tip in combination with InnuPure® C16/C16 *touch*. The automated procedure starts with the adsorption of nucleated blood cells to the Smart Modified Surfaces. Following lysis, the lysates are used for automated extraction of high molecular weight genomic DNA.

After washing the genomic DNA is eluted from the Smart Modified Surfaces and is ready for use in subsequent downstream applications.

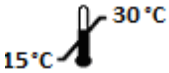
The combination of patented, low-salt DC-Technology® with patent-pending Smart Modified Surface is optimized to get a maximum of yield and quality.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual

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

Symbol	Information
	**REF** Catalogue number.
 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 number** The number of the kit charge.
	**Manufactured by** Contact information of manufacturer.
	**For single use only** Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p. 4).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully prior to 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, which are shown.

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!
Don't 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 potential infectious human samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

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, please ask for the material safety data sheets (MSDS's).

3 Storage conditions

Store lyophilized **Proteinase K** at 4 °C to 8 °C. Divide dissolved **Proteinase K** into aliquots and storage at -22 °C to -18 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!

All other components of the smart Blood DNA Midi Direct prep (a) 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 have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming. For further information see chapter "Kit components"(→ p. 8).

4 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the smart Blood DNA Midi Direct prep (a) or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use” p. 3) (→ “Product specifications” p 13). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols than those described below. Analytik Jena AG kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalent regulations required in other countries.

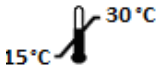
All products sold by the Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
For research use only!

6 Kit components

	Σ 16	Σ 96
REF	845-AS[S/P]-3008016	845-AS[S/P]-3008096
SmartExtraction Tips	16	96
Proteinase K	for 1 x 1.5 ml working solution	for 4 x 1.5 ml working solution
Reagent Strip N* (* Depending on order)	16 (pre-filled, sealed)	96 (pre-filled, sealed)
Reagent Plate N* (* Depending on order)	2 (pre-filled, sealed)	12 (pre-filled, sealed)
Filter Tips	1 x 16	1 x 96
Elution Tubes (0.65 ml)	16	2 x 48
Elution Caps (Stripes)	2	12
Manual	1	1
Initial steps	**Proteinase K** Dissolve by addition of **1.5 ml** of **ddH_2O**, mix thoroughly and store as described below.	**Proteinase K** Dissolve by addition of **1.5 ml** of **ddH_2O**, mix thoroughly and store as described below.

IMPORTANT
Store lyophilized **Proteinase K** at 4 °C to 8 °C! Divide dissolved **Proteinase K** into aliquots and storage at -22 °C to -18 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!



STORAGE CONDITIONS
All other components are stored at room temperature.

Components not included in the kit

- Sterile ddH_2O for dissolving **Proteinase K** and filling up the samples less than 0.5 ml or 1.0 ml whole blood
- 1.5 ml reaction tubes
- 2.0 ml reaction tubes

7 GHS Classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Reagent Plates/Strips N	Propan-2-ol 50–100 % Ethanol 50–100 % Calcium chloride 10–25 % Sodium dodecyl sulphate 2.5–≤10 % Polyethylene glycol octylphenol ether 0.25–≤2.5 % Hydrochloric acid 0.1–≤2.5 % Acetic acid 2.5–≤10 %	 Danger	225; 290, 319; 336	101;102;103;210;241;303+361+353;305+351+338;405;501
Proteinase K	Proteinase, engyodontium album	 Danger	315, 319, 334, 317, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501

7.1 Hazard phrases

225 Highly flammable liquid and vapor.

290 May be corrosive to metals.

315 Causes skin irritation.

317 May cause an allergic skin reaction.

319 Causes serious eye irritation.

334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

335 May cause respiratory irritation.

336 May cause drowsiness or dizziness.

7.2 Precaution phrases

101	If medical advice is needed, have product container or label at hand.
102	Keep out of reach of children.
103	Read label before use.
210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
241	Use explosion-proof electrical/ventilating/lighting equipment.
261	Avoid breathing dust/fume/gas/mist/vapors/spray.
280	Wear protective gloves/protective clothing/ eye protection/face protection.
362	Take off contaminated clothing.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
302+352	IF ON SKIN: Wash with plenty of water.
303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
403+233	Store in a well-ventilated place. Keep container tightly closed.
305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

8 Product specifications

1. Starting material:
   - 0.2–1 ml whole blood (fresh or frozen) stabilized with EDTA, citrate or heparin.
   - 0.2–1 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.

NOTE

Fresh means maximal storage time of 24 h at room temperature followed by a maximum storage time of 6 days at 4 °C to 8 °C.

Frozen 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:
   - Lysis — external lysis steps are not required
   - Extraction — depends on extraction device and protocol of choice. Duration of extraction protocols is specified in the relevant chapters.
3. Typical yields:

Whole blood volume	Typical yield
0.5 ml	5–15 µg
1.0 ml	15–40 µg

Using buffy coat of an equivalent of 2 ml whole blood, 30–60 µg DNA are expected.

NOTE

Yield of isolated DNA is affected by sample condition. The sample condition depends on storage conditions as well as on 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!

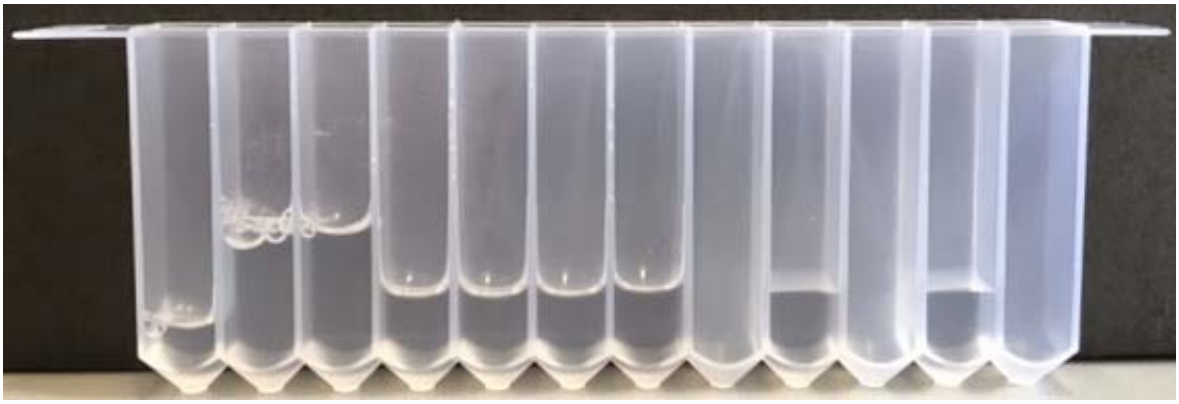
9 Preparation of buffy coat from whole blood

The following instructions can be used for the preparation of buffy coat.

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. Don't disturb the interphase!
4. Carefully aspirate the interphase and transfer into a new 1.5 ml tube.

10 Preparation of Reagent Plates or Reagent Strips

10.1 General filling scheme



Cavity 1:	Lysis Solution	Cavity 7:	Washing Solution
Cavity 2:	Cell Binding Solution	Cavity 8:	Empty
Cavity 3:	Cell Binding Solution	Cavity 9:	Elution Buffer
Cavity 4:	Binding Solution	Cavity 10:	Empty
Cavity 5:	Washing Solution	Cavity 11:	Washing Solution
Cavity 6:	Washing Solution	Cavity 12:	Empty

10.2 Unpacking of Reagent Plates or Strips and piercing of sealing foil

NOTE
According to transport regulations Reagent Reservoirs are wrapped into plastic bags only when transported by airplane.

A Unpacking of Reagent Reservoirs

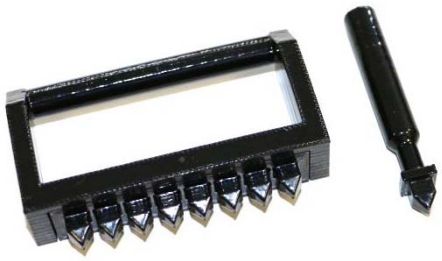
Reagent Reservoirs are optional delivered wrapped into plastic bags for transport protection.

Carefully open the overpack of Reagent Reservoirs by using scissors.

B Piercing of sealing foil

NOTE
Invert the Reagent Plates or Reagent Strips 3–4 times and thump it onto a table to collect the pre-filled solutions at the bottom of the wells.

Before using Reagent Plates or Reagent Strips the sealing foil has to be pierced manually. Always wear gloves while piercing of the foil!

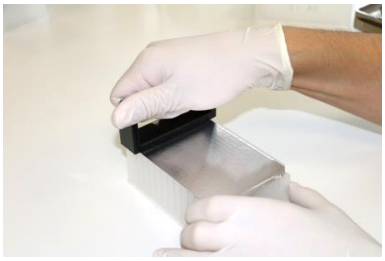
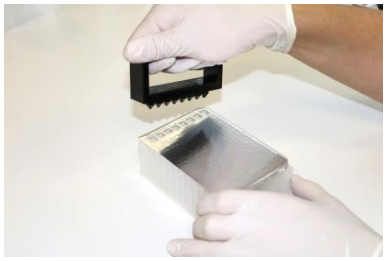
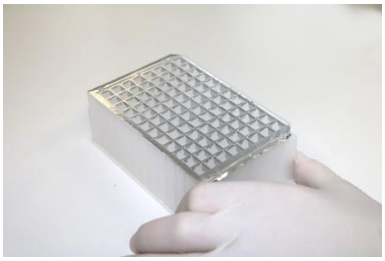


Reagent Plates or Reagent Strips are pre-filled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (single piercer or 8fold piercer).

Keep the Reagent Plates or Reagent Strips in a horizontal position to avoid spilling of the reagents while piercing of the foil.

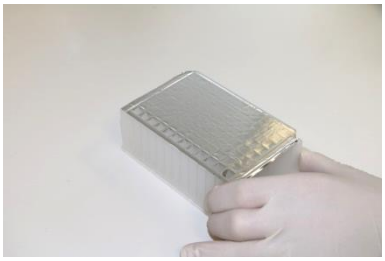
Open all cavities (one row per sample).

Using 8 samples in parallel

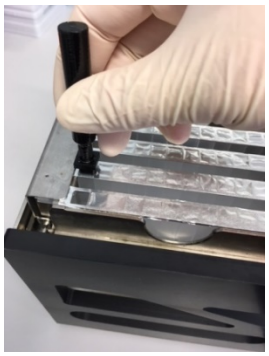
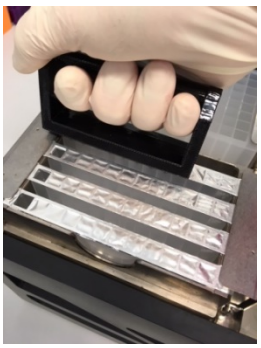




Using single samples





Using stripes


NOTE!

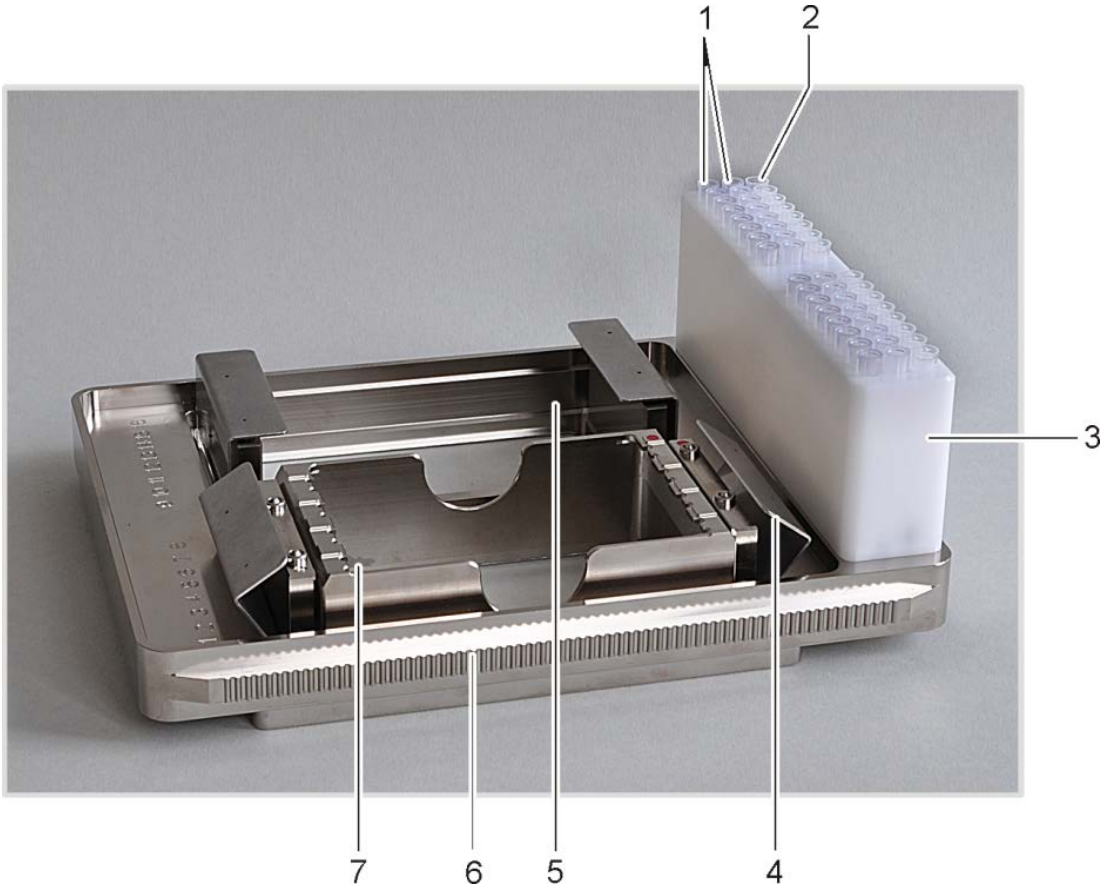
The sample will be processed using a liquid handling platform. Please follow the instruction of the manual according to the following chapters:

Automated extraction using InnuPure® C16/C16 *touch* on p. 19

Pay special attention to sub-chapter “Loading the sample to InnuPure® C16/C16 *touch*”.

11 Automated extraction using InnuPure® C16/C16 *touch*

11.1 Sample tray of InnuPure® C16/C16 *touch*



No. 1:	SmartExtraction and standard filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Pressure pad
No. 5:	Sample block for reagent plates or adapter for reagent strips
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for reagent strips

11.2 Preparing sample tray of InnuPure® C16 / C16 *touch*

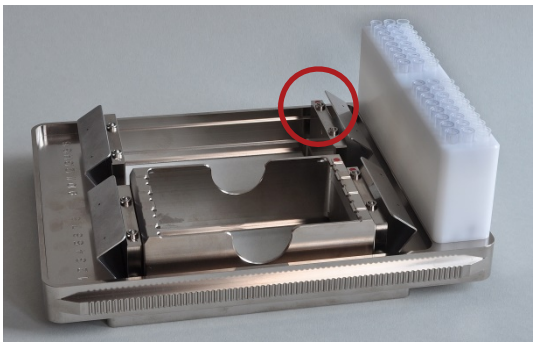
NOTE

The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more Reagent Strips as number of samples!

1. Move the InnuPure® C16/C16 touch sample tray into the priming station and fold the holding-down clamp at the sample tray upwards!
2. Place the Reagent Plate or an adapter for the Reagent Strips into the holder of the sample tray. Using Reagent Plates, the notched corner of the Reagent Plate has to align with the colored dot at the holder. Using adapters and Reagent Strips, the colored dot of the adapter has to align with the colored dot at the holder and Reagent Strips have to be inserted in a way that the “AJ” labels are arranged at the side of the adapter, which is more distant from the tip block.

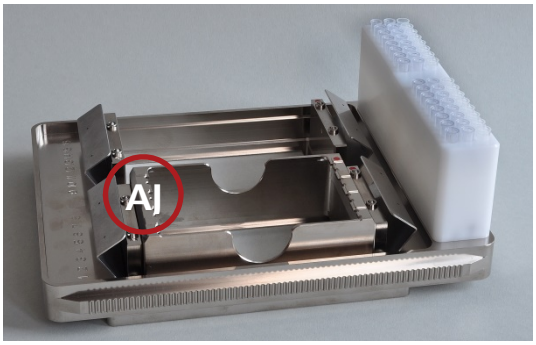
Reagent Plate

The notched corners of the Reagent Plate must point to the colored dot on the holder.



Reagent Strips

Place the Reagent Strips into the adapter. The long tab marked with the label "AJ" must point to the side of the adapter which is more distant from the tip block.



CAUTION

Both holders have to be equipped with a Reagent Plate or Reagent Strips. If applicable use an empty or dummy plate for the respective holder.

3. Fold down the holding-down clamp to prevent the Reagent Plates and Reagent Strips to be pulled out of the holder during the extraction process.
4. For each extracted sample place a SmartExtraction Tip and a filter tip in the smaller drill holes of the tip block (→ "Handling of SmartExtraction Pipette Tips" p. 22)

NOTE

Extracted high molecular weight DNA from large sample amounts tends to be very viscous. In order to improve the handling of DNA for downstream applications which don't require high molecular weight DNA, extraction protocols include a homogenization step reducing the fragment size of extracted DNA. If downstream application requires high molecular weight DNA, Tip row 2 of the tip block should be left empty and not be equipped with standard filter tips (→ "Handling of SmartExtraction Pipette Tips" p. 22). As a result, the eluate will remain in **cavity 12** of the Reagent Plastic at the end of the protocol. Transfer of the eluate into storage tubes (e.g. Elution Tubes with Elution Caps, 1.5 ml reaction tubes) has to be done manually. In order to avoid a loss of DNA integrity pipet carefully with a wide-bore or cut tip.

5. Place the Elution Tubes into the wider drill hole at the edge of the tip block. Empty sample positions do not need to be filled.

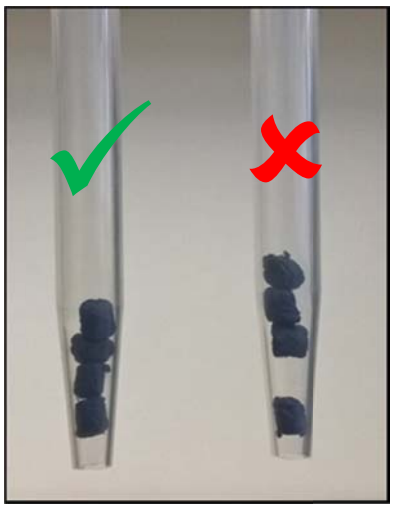
NOTE

Especially with the Reagent Strips make sure that for every Reagent Strip the tips and the elution vessel are in the corresponding positions in the tip block!

IMPORTANT NOTE

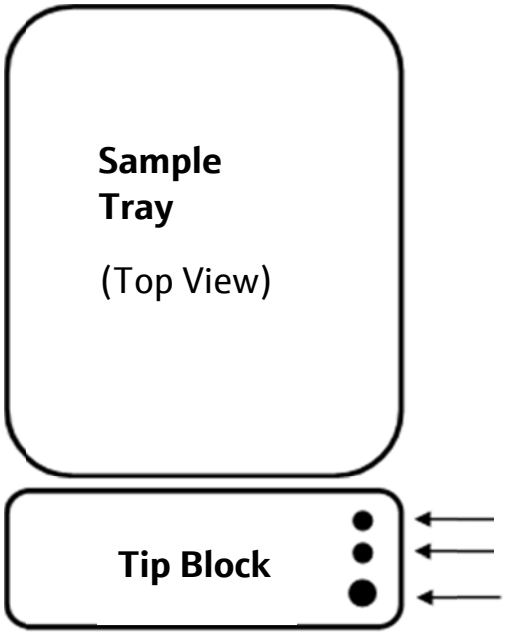
Use Elution Tubes (0.65 ml) with corresponding Elution Caps.

11.3 Handling of SmartExtraction Pipette Tips



Checking the SmartExtraction tips.

Make sure that the Smart Modified Material is collected near the outlet of the SmartExtraction Tip. If necessary flip the tip by finger or edge of table or invert it a few times. The optimal position of the Smart Modified Material inside the tip is shown in the picture on the left side.



Loading Pipette Tips to InnuPure® C16/C16 *touch*.

The SmartExtraction Tips are inserted in the tip row 1. The tip row 1 is the tip row adjacent to the Reagent Plates or Reagent Strips. See figure left.

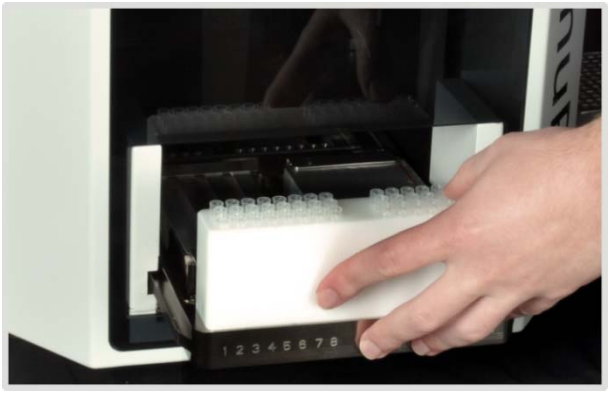
11.4 Loading the sample to InnuPure® C16/C16 *touch*

1. Transfer **50 µl** of **Proteinase K** into the **first cavity** of Reagent Strips or Reagent Plates.
2. Transfer the **blood sample or buffy coat and water** according to the table below into the **specified cavity** of Reagent Strips or Reagent Plates. Please pay attention to transfer the blood sample first and then transfer the water.

Total sample volume	Sample		Water	
	Cavity 2	Cavity 3	Cavity 2	Cavity 3
0.2 ml	0.2 ml	-	0.3 ml	-
0.3 ml	0.3 ml	-	0.2 ml	-
0.4 ml	0.4 ml	-	0.1 ml	-
0.5 ml	0.5 ml	-	-	-
0.6 ml	0.3 ml	0.3 ml	0.2 ml	0.2 ml
0.7 ml	0.35 ml	0.35 ml	0.15 ml	0.15 ml
0.8 ml	0.4 ml	0.4 ml	0.1 ml	0.1 ml
0.9 ml	0.45 ml	0.45 ml	0.05 ml	0.05 ml
1.0 ml	0.5 ml	0.5 ml	-	-

11.5 Starting the InnuPure® C16

1. Switch on the InnuPure® C16 and wait for the device initialization to complete, which is signaled by a beeping sound.
2. Move the loaded sample tray with the reagent strips forward into the adapter on the front of the InnuPure® C16. The serrated rails at the side of the sample tray must protrude into the grooves of the adapter. After pressing lightly against the tip block the sample tray is automatically pulled into the device.



Important – Caution
Risk of crushing
Immediately let go of the sample tray once it is being pulled in. Otherwise there is a risk of your hand being crushed.

6. Start the extraction protocol:
   - Press [SELECT PROTOCOL] in the starting window.
   - Select the desired extraction protocol and the for your application fitting wash procedure.
   - Select for up to **500µl sample volume:**

     "SE_Blood_Midi_direct_500_dry_C16_03"

     and press [START].

     or for up to **1,000µl sample volume:**

     "SE_Blood_Midi_direct_1000_dry_C16_03"

     and press [START].

7. Enter elution volume and press [OK].

Volume of whole blood sample	Recommended elution volume
Up to 0.5 ml	min. 200 µl
0.6–1.0 ml	300–500 µl

   Using buffy coat minimum recommended elution volume is 300 µl.

8. If needed, choose log file and enter sample ID's, press [OK] or [CANCEL].

NOTE
It is possible to enter sample ID's and to create a run log file. Find more detailed information how to start an extraction protocol using InnuPure® C16 in the user manual "6.3.5 Using the sample setup tool" on page 37!

9. After completion of the protocol press [NEXT] and the sample tray is then automatically moved out of the device.

NOTE
The chosen protocol is performed by the device and after the protocol is finished, the tray with the purified samples will be moved out after pressing [NEXT] and the message 'Program finished' is shown on the screen of the device!

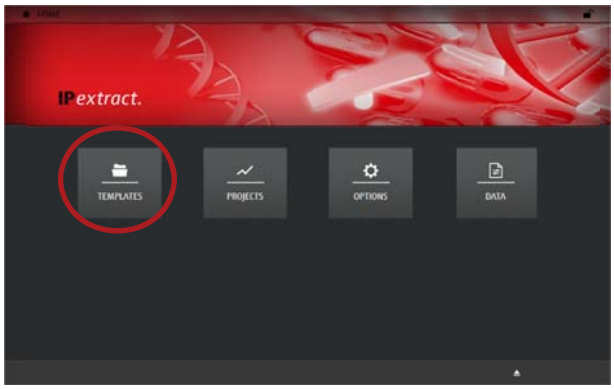
10. Remove the sample tray from the adapter of the InnuPure® C16 and move it back into the priming station.
11. After finishing the extraction protocol, the Elution Tubes (0.65 ml) contain the extracted DNA. Close the lids and store the DNA under proper conditions.

NOTE
Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

11.6 Starting the InnuPure® C16 *touch*

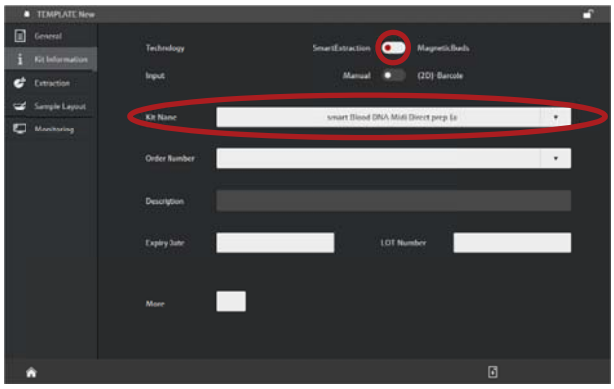
NOTE
The following instructions describe the necessary steps for the start of the InnuPure® C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure® C16 *touch*.

1. Switch on the InnuPure® C16 *touch* and the tablet computer. Wait until the home screen of IP*extract* is displayed on the tablet screen.



NOTE
Home screen of IP*extract*

2. Choose [TEMPLATES]→[New Template]→[Kit-based].
3. Enter optional information in the tab “General”.
4. Choose the tab “Kit Information” and switch the “Technology” to “SmartExtraction”!
5. Choose your desired kit from “Kit Name”!

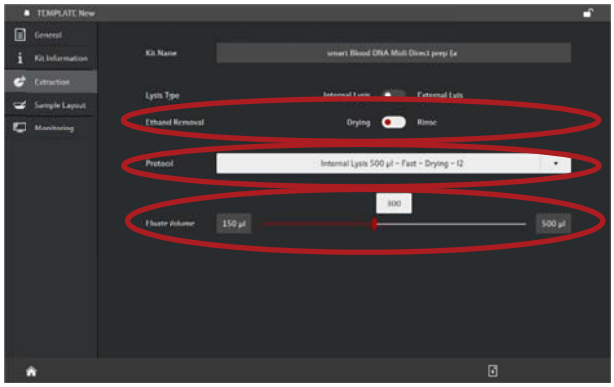


NOTE
“Kit Information” tab

6. Enter optional information in the tab “Kit Information”
7. Choose the tab “Extraction” and choose the desired method for “Ethanol Removal” and “Protocol”.

   “Drying” – Ethanol is removed by evaporation

   “Rinse” – Ethanol is washed away using a special Washing Solution



NOTE
“Extraction” tab

Extraction procedure	Protocol on InnuPure® C16 *touch*
Starting volume: up to 500 µl	Internal Lysis 500 µl – Drying – 03 (88 min) Internal Lysis 500 µl – Rinse – 03 (85 min)
Starting volume: 500 µl – 1000 µl	Internal Lysis 1000 µl – Drying – 03 (121 min) Internal Lysis 1000 µl – Rinse – 03 (117 min)

NOTE
For most applications Ethanol Removal by “Drying” is recommended. If the extracted DNA is conceived for very ethanol-sensitive downstream applications (e.g. Droplet PCR), chose the option “Rinse”.

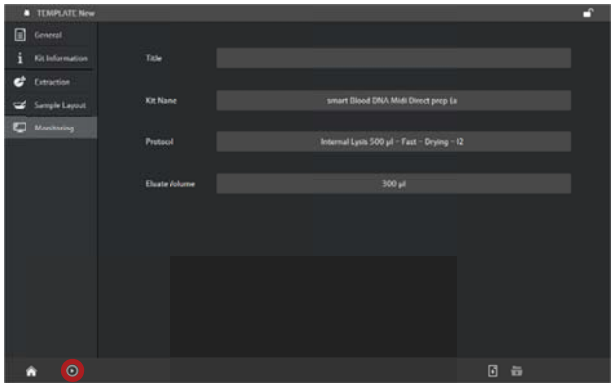
“Rinse” can also be selected for time-sensitive preparations, since the protocol saves approx. 4 minutes, but the yield might be significantly lower.

12. Adjust your desired “Eluate Volume“ using the slider or the text field. Recommended elution volumes are listed in the table below.

Volume of whole blood sample	Recommended elution volume
Up to 0.5 ml	min. 200 µl
0.6–1.0 ml	300–500 µl

Using buffy coat minimum recommended elution volume is 300 µl.

13. Choose the tab “Monitoring”, control the settings and start the protocol by tapping the start button.



NOTE
“Monitoring” tab

14. Follow the instructions displayed on the tablet screen.
15. Completion of the protocol is indicated by a message on the tablet screen. Follow the instructions on the screen to remove the sample tray from the device.
16. The Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

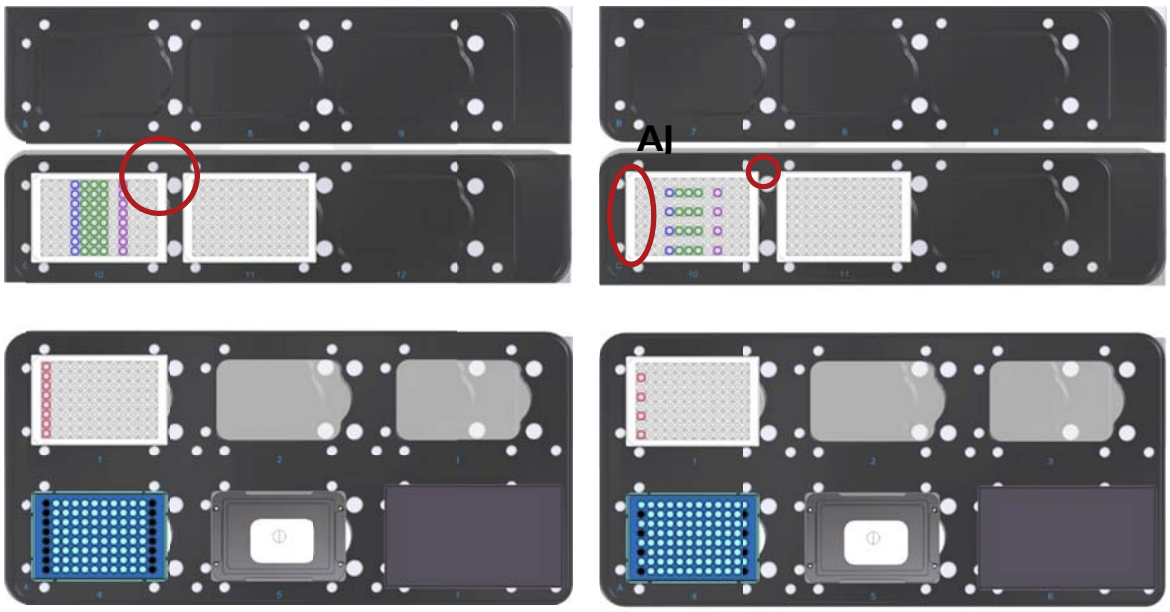
NOTE
Store the DNA and RNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

12 Automated extraction using CyBio® FeliX

12.1 Accessories needed

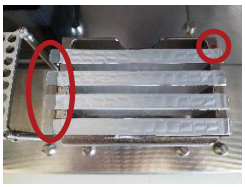
- CyBio® FeliX Basic Unit with Enclosure and CyBio® Composer Software (OL5015-24-100)
- CHOICE™ Head (OL3316-11-300) and 8-Channel CHOICE™ 10 µl – 1000 µl Adapter (OL3316-11-330)
- alternatively CyBio® FeliX Head R 96/1000 µl (OL3316-14-950) and 8-Channel Adapter; Head R 96 (OL3317-11-330)
- Tip Rack 96/1000 µl (OL3317-11-140)
- Waste Box I (small) (844-00430-0)
- Waste Bag (10-406-342)
- BioShake 3000-T elm (QINSTRUMENTS-2016-0517)
- Mounting Kit; BioShake 3000 Series (OL3317-23-692)
- Adapter for Deepwell Plates (QINSTRUMENTS-2016-1214)
- DeepWell Plate for SmartExtraction Kits (845-FX-8500025)
- System Specific Pre-configured Desktop Computer (0006100-00)
- Optional: Elution Plate (1.2 ml) for InnuPure C96, 5 pieces (845-IP-0096005)
- Optional: Adapter for Reagent Strips (845-60006-0)

12.2 Preparing CyBio® FeliX



Deck layout using **Reagent Plate**

Deck layout using **Reagent Strips**



1. Place the empty **Waste Box** onto deck **position 6**.
2. Place the **8-Channel Adapter** with the **37 mm Support** onto **deck position 5** (the adapter to use depends on the pipetting head).
3. Place the **Tip Rack 96/1000 µl** onto **deck position 4**.
4. Place one **SmartExtraction Tip** per sample to **column 1** and one **Filter Tip** per sample to **column 12** of the **Tip Rack 96/1000 µl.**

NOTE

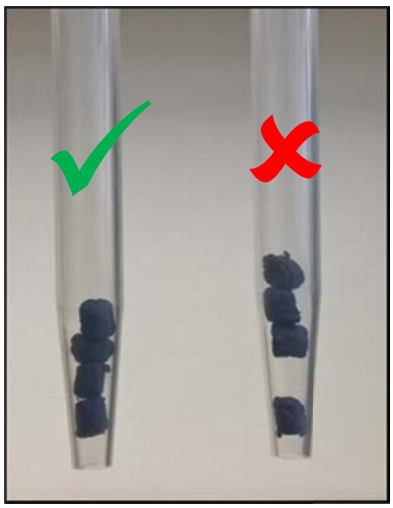
Extracted high molecular weight DNA from large sample amounts tends to be very viscous. In order to improve the handling of DNA for downstream applications, which don't require high molecular weight DNA, extraction protocols include a homogenization step reducing the fragment size of extracted DNA. If downstream application requires high molecular weight DNA, column 12 of the Tip Rack 96/1000 µl must be left empty. As a result, the eluate will remain in **column 12** of the DeepWell Plate for SmartExtraction Kits (deck position 1) at the end of the protocol. Transfer of the eluate into storage tubes (e.g. Elution Tubes with Elution Caps, 1.5 ml reaction tubes) has to be done manually. In order to avoid a loss of DNA integrity pipet carefully with a wide-bore or cut tip.

NOTE

Especially with the Reagent Strips make sure that for every Reagent Strip the tips are in the corresponding positions in the Tip Rack 96/1000 µl!

5. As a final Elution Plate (**deck position 11**) multiple options are possible:
   -Elution Plate (1.2 ml) for InnuPure C96, 5 pieces (845-IP-0096005)
   -Micronic 750 µl pre-capped and racked 2D-tubes (MP52706-Y20)
   -Greiner Cryo.S 600 µl pre-racked (977561, 977580)

12.3 Handling of SmartExtraction Tips



Checking the SmartExtraction Tips.
Make sure that the Smart Modified Material is collected near the outlet of the SmartExtraction Tip. If necessary, flip the tip by finger or edge of table or invert it a few times. The optimal position of the Smart Modified Material inside the tip is shown in the picture on the left side.

12.4 Loading the sample and starting CyBio® FeliX

1. Transfer **50 µl** of **Proteinase K** into the **first cavity** of Reagent Strips or **first column** of Reagent Plate.
2. Transfer the **blood sample or buffy coat and water** according to the table below into the **specified cavity** of Reagent Strips or Reagent Plate. Please pay attention to transfer the blood sample first and then transfer the water.

Total sample volume	Sample		Water	
	Cavity 2	Cavity 3	Cavity 2	Cavity 3
0.2 ml	0.2 ml	-	0.3 ml	-
0.3 ml	0.3 ml	-	0.2 ml	-
0.4 ml	0.4 ml	-	0.1 ml	-
0.5 ml	0.5 ml	-	-	-
0.6 ml	0.3 ml	0.3 ml	0.2 ml	0.2 ml
0.7 ml	0.35 ml	0.35 ml	0.15 ml	0.15 ml
0.8 ml	0.4 ml	0.4 ml	0.1 ml	0.1 ml
0.9 ml	0.45 ml	0.45 ml	0.05 ml	0.05 ml
1.0 ml	0.5 ml	0.5 ml	-	-

NOTE
The needed number Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more Reagent Strips as number of samples!

3. Place the opened Reagent Plate or optionally one Strip Adapter with Reagent Strips onto **deck position 10.**
4. Place the DeepWell Plate for SmartExtration Kits (845-FX-8500025) on the BioShake T-elm 3000 at **deck position 1.**

NOTE
For Reagent Plates the notched corners of the Reagent Plate must be oriented to the upper corners of deck position 10. For Reagent Strips, put the Strip Adapter on deck position 10 in a way that the red dot of the adapter resides at the rear right corner of deck position 10. Put in the Reagent Strips in a way that the AJ labels are oriented to the left side of the adapter (→ “Preparing CyBio® FeliX”, p. 30).

5. Switch on the CyBio® FeliX and open Composer.

6. Load the extraction protocol:
   SE_Blood Midi Direct_500_dry_FX_8_01 (90 minutes) or
   SE_Blood Midi Direct_1000_dry_FX_8_01 (120 minutes)
7. Adjust parameter elution volume ($(Vol_elute)) in the headline of the protocol; press [OK] and start the protocol. With a sample volume of up to 500 µl whole blood we recommend to set the elution volume at least to 200 µl. With buffy coat as a sample we recommend a minimum elution volume of 300 µl.

NOTE
The chosen protocol is performed by device and after the protocol is finished the message “Purification process completed” is shown in the screen of the computer!

8. After finishing the extraction protocol, the eluate will be in the final elution plate in column 1 on **deck position 11**. If high molecular weight DNA is required and consequently standard 1 ml filter tips were not placed in Tip Rack 96/1000 µl, the eluate will remain in column 12 of the DeepWell Plate for SmartExtraction Kits on **deck position 1**.
9. After finishing the extraction protocol, remove and discard the used Deep Well Plates and the used tips.

NOTE
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 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Smart Modified Material not collected near the tip opening	Flip the Pipette Tip by finger or edge of table or invert the Pipette Tip a few times to collect Granulates at the lower part of pipette tip.
High viscosity extracted DNA	
Insufficient amount of Elution Buffer	Repeat your experiment and 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

14 Related Products

Name	Amount	Order No.
Additional products for nucleic acid purification		
innuPREP Proteinase K	6 mg	845-CH-0010006
	30 mg	845-CH-0010030
Automated nucleic acid purification		
smart DNA prep (a)	16 rxn (Strips)	845-ASS-2008016
	96 rxn (Strips)	845-ASS-2008096
	16 rxn (Plates)	845-ASP-2008016
	96 rxn (Plates)	845-ASP-2008096
smart Blood DNA Midi prep (a)	16 rxn (Strips)	845-ASS-1208016
	96 rxn (Strips)	845-ASS-1208096
	16 rxn (Plates)	845-ASP-1208016
	96 rxn (Plates)	845-ASP-1208096

engl. 02/18 – Analytik Jena AG, Jena

Headquarters

Analytik Jena AG
Konrad-Zuse-Str. 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279
info@analytik-jena.com
www.analytik-jena.com

Pictures: Analytik Jena AG
Subject to changes in design and scope of delivery as well as further technical development!