Instructions for Use
Life Science Kits & Assays

smart Blood DNA Midi Direct prep (a)

iST Innuscreen GmbH

Order No.:

845-ASS-3008016 16 reactions
845-ASP-3008016 16 reactions
845-ASS-3008096 96 reactions
845-ASP-3008096 96 reactions

Publication No.: HB_ASP-3008_e_220328

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Contents

1 Introduction

1.1 Intended use

The smart Blood DNA Midi Direct prep (a) kit 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 invented by IST Innuscreen GmbH.

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

3 Storage conditions

The kit is 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 Direct prep (a)” 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 have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

4 Functional testing and technical assistance

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

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the “smart Blood DNA Midi Direct prep (a)” or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 30 9489 3380. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications” p. 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 the IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 16	Σ 96
REF	845-ASS-3008016[a] 845-ASP-3008016[b]	845-ASS-3008096[a] 845-ASP-3008096[b]
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[a] (* Depending on order)	16 (pre-filled, sealed)	96 (pre-filled, sealed)
Reagent Plate N[b] (* Depending on order)	2 (pre-filled, sealed)	12 (pre-filled, sealed)
Filter Tips	16	96
Elution Tubes (0.65 ml)	16	2 x 48
Elution Caps (Stripes)	2	12
Manual	1	1

6.2 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 m and 2.0 ml reaction tubes

6.3 Related products

- Piercing Tool, Set (contains Single Piercer and 8 well Piercer; 845-PTS-0000002, Analytik Jena GmbH, Jena, Germany)

7 Initial steps before starting

- Add 1.5 ml ddH2O to each vial of **Proteinase K**, mix thoroughly and store as described above.

- Invert the Reagent Plate / Reagent Strips for 3–4 times and thump it onto a table to collect the prefilled solutions at the bottom of the wells.

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!

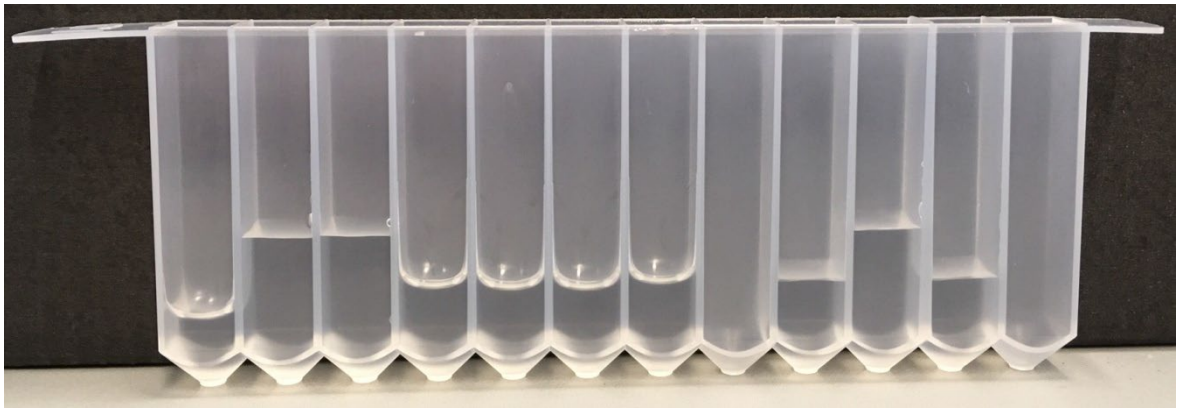
9 Preparation of buffy coat from whole blood

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.

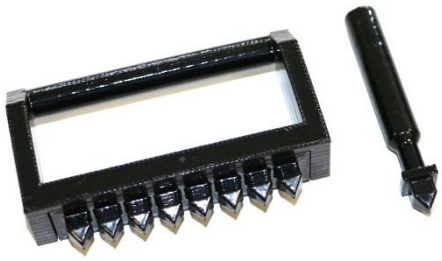
A Unpacking of Reagent Reservoirs

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 / 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!

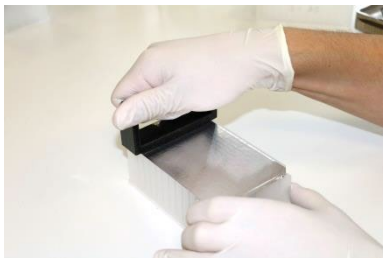
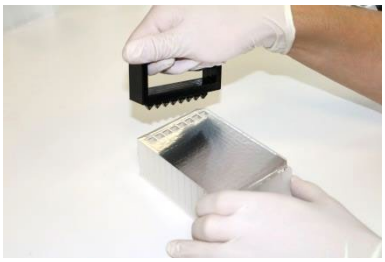
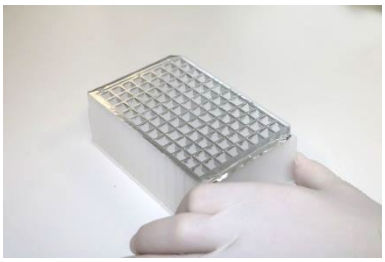


Reagent Plates / Reagent Strips are pre-filled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (single piercer or 8fold piercer).

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

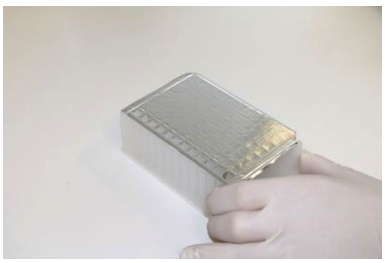
IMPORTANT NOTE
Open all cavities (one row per sample)!

Using 8 samples in parallel

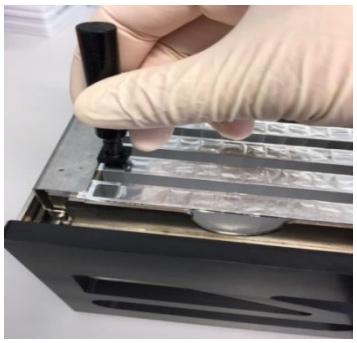
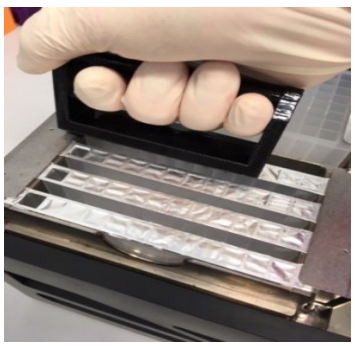
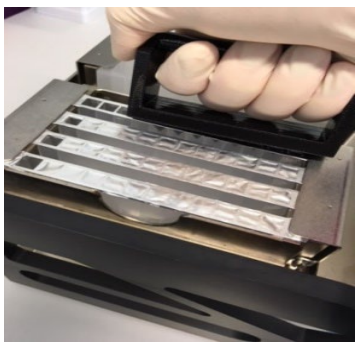




Using single samples





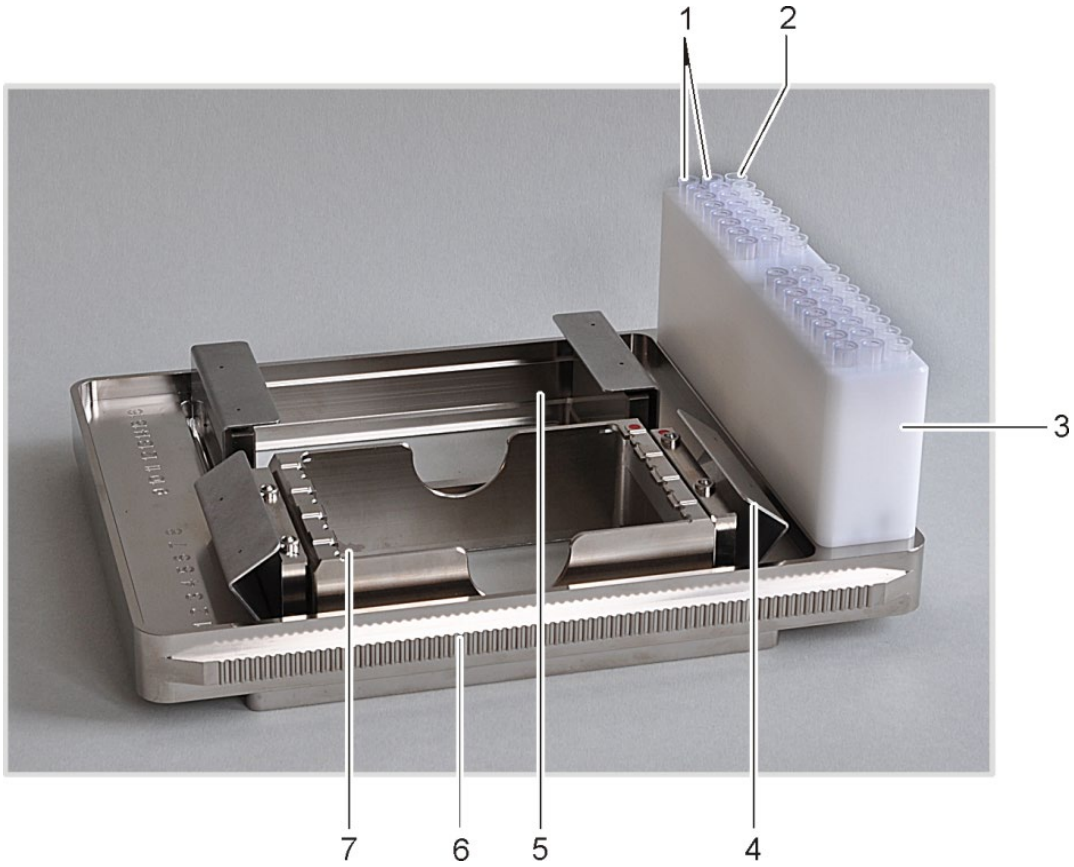
Using stripes

NOTE
The sample will be processed using the InnuPure C16 / C16 touch.
(→ “Automated extraction using InnuPure C16 / C16 *touch*”, p.12)

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

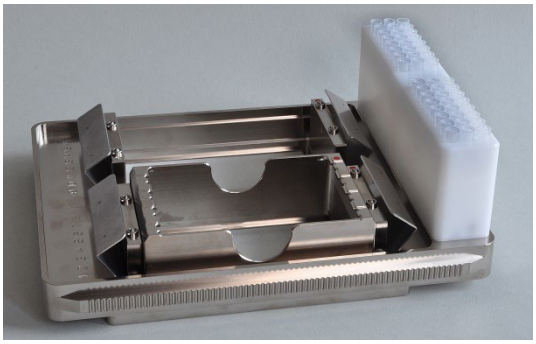
11.2 Preparing sample tray of InnuPure C16 / C16 touch

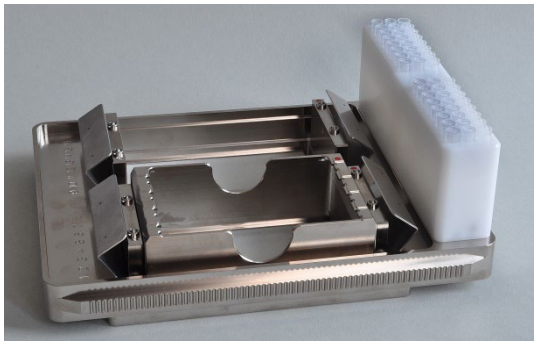
NOTE
The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more Reagent Strips as number of samples!

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.

t 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. 15).

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. 15). 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.

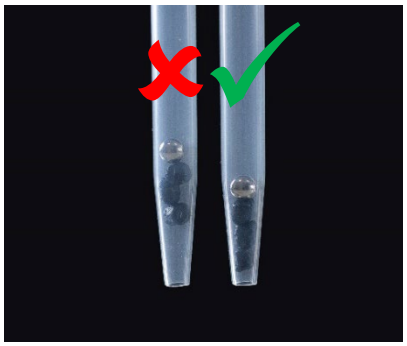
NOTE

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

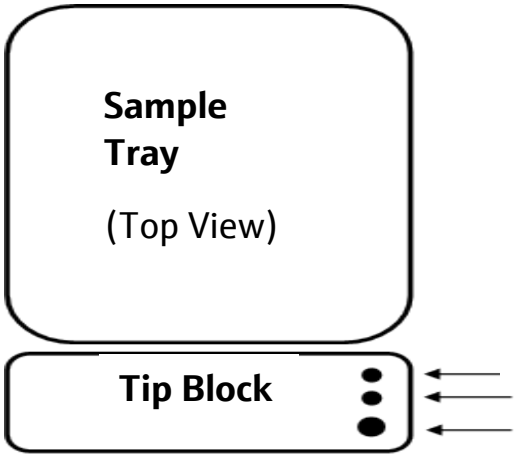
Use Elution Tubes (0.65 ml) with corresponding Elution Caps.

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

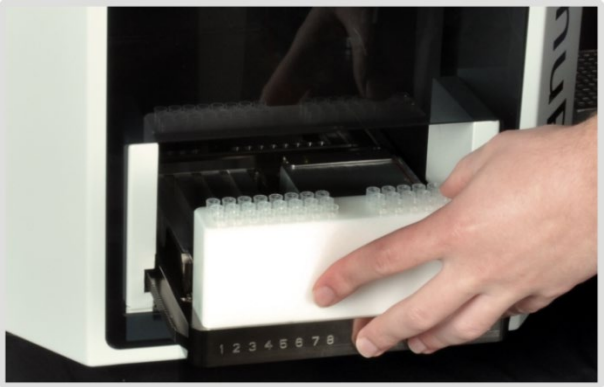
1. Transfer **50 µl** of **Proteinase K** into the **first cavity** of Reagent Strips or Reagent Plates.
2. Transfer the **blood sample or buffy coat and water** according to the table below into the **specified cavity** of Reagent Strips or Reagent Plates. Please pay attention to transfer the blood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

	Cavity 2	Cavity 3	Cavity 2	Cavity 3
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.

3. 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].

4. 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**.

5. 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!

6. 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!

7. Remove the sample tray from the adapter of the InnuPure C16 and move it back into the priming station.
8. 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.

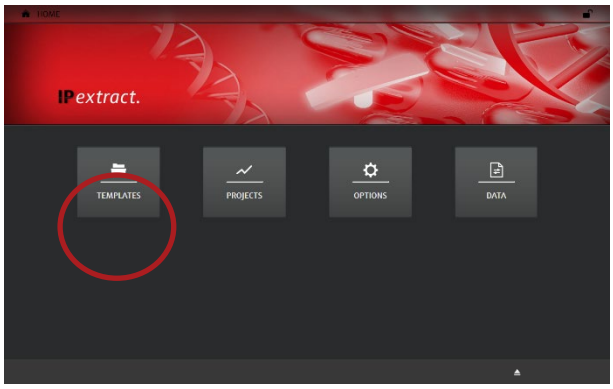
NOTE
Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

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.

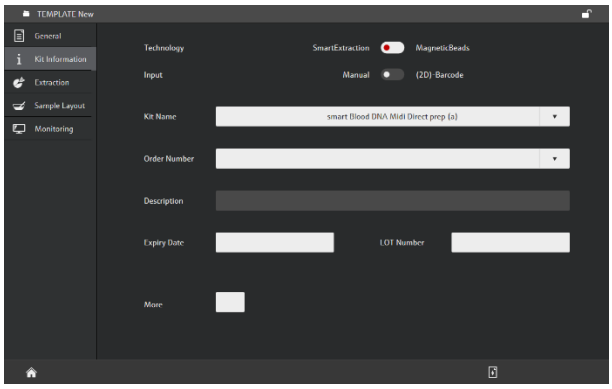
opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

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”!

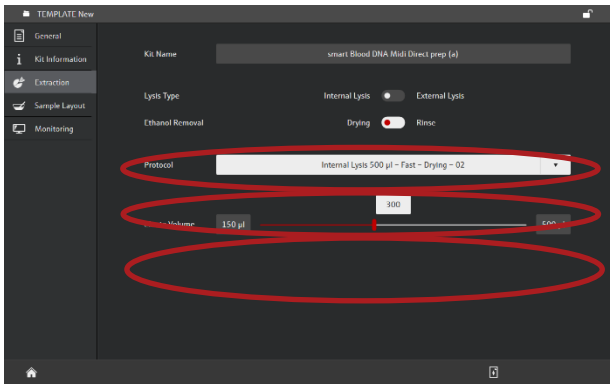


NOTE
“Kit Information” tab

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)

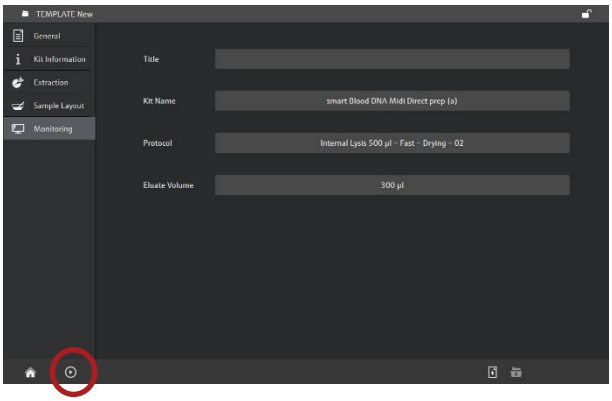
NOTE
For most applications Ethanol Removal by **"Drying"** is recommended. If the extracted DNA is conceived for very ethanol-sensitive downstream applications (e.g. Droplet PCR), chose the option "Rinse". **"Rinse"** can also be selected for time-sensitive preparations, since the protocol saves approx. 4 minutes, but the yield might be significantly lower.

8. 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.

9. Choose the tab "Monitoring", control the settings and start the protocol by tapping the start button.



NOTE
"Monitoring" tab

10. Follow the instructions displayed on the tablet screen.
11. 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.
12. 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 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. Elute DNA with 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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