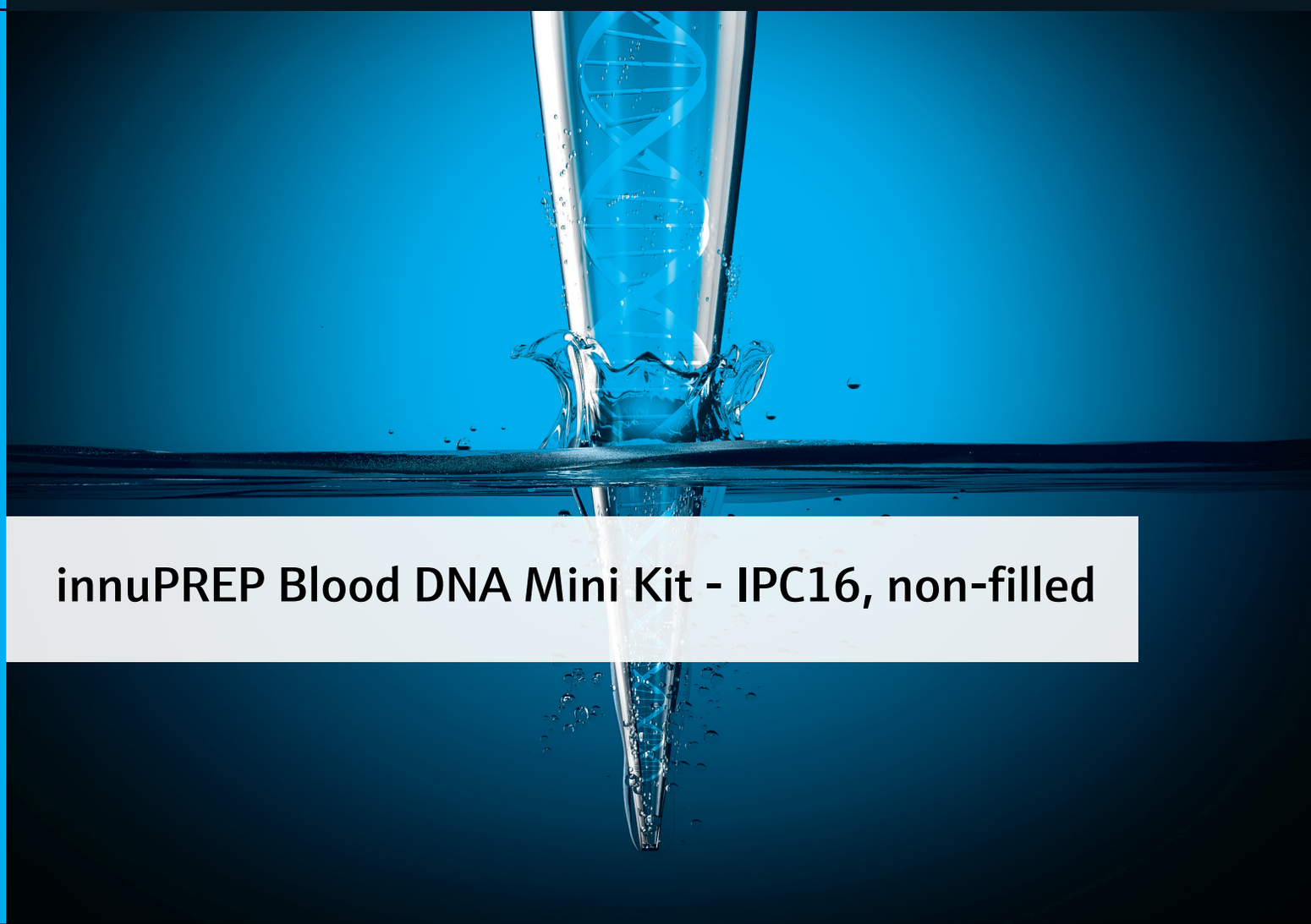
Instructions for Use
Life Science Kits & Assays



innuPREP Blood DNA Mini Kit - IPC16, non-filled

Innuscreen GmbH

Order No.:
845-PPP-1116016 16 reactions
845-PPP-1116096 96 reactions
845-PPP-1116480 480 reactions

Publication No.: HB_PPP-1116_e_220927

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Contents

1 Introduction

1.1 Intended use

The **innuPREP Blood DNA Mini Kit – IPC16, non-filled** has been designed for the fully automated isolation of genomic DNA from 200 µl to 400 µl of fresh or frozen whole blood sample (EDTA, citrate or heparin stabilized). The extraction procedure is based on a new patented chemistry.

All steps of the extraction process are fully automated and run completely on the InnuPure C16 / C16 *touch*. The MAG Suspension F and the whole blood sample are transferred without any external handling steps into the Reagent Plate of the kit, which must be prefilled with all reagents needed for the extraction process. Upon addition of Proteinase K, the extraction procedure begins with sample lysis, followed by binding of DNA to surface-modified magnetic particles. After several washing steps the DNA is eluted from the magnetic particles with RNase-free water and is ready to be used in downstream applications. The extraction chemistry in combination with the InnuPure C16 / C16 *touch* protocol is optimized to get maximum 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 and the kit", 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. IST Innuscreen GmbH has not tested the liquid waste generated during usage of 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 the 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 **MAG Suspension F** and lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

All other components of the **innuPREP Blood DNA Mini Kit – IPC16, non-filled** 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, these can be dissolved 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 this manual. This kit was produced 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 **innuPREP Blood DNA Mini Kit – IPC16, non-filled** or other IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information in Germany please contact info.innu@ist-ag.com. For support in 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 material than those referred to in the manual (→ “Product specifications”, p. 9). Since the performance characteristics of IST Innuscreen GmbH kits have only been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits when 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	Σ 480
REF	845-PPP-1116016	845-PPP-1116096	845-PPP-1116480
MAG Suspension F	0.25 ml	1.1 ml	5 × 1.1 ml
Proteinase K	For 3 × 0.3 ml working solution	For 4 × 1.5 ml working solution	For 17 × 1.5 ml working solution
Deep Well Plate (2.0 ml)	2 (empty)	12 (empty)	60 (empty)
Filter Tips	2 × 16	2 × 96	10 × 96
Elution Tubes (0.65 ml)	16	2 × 48	10 × 48
Elution Caps (Stripes)	2	12	5 × 12
Elution Strips	2	12	5 × 12
RNase-free Water	30 ml	2 x100 ml	4 x 200 ml
Lysis Solution CBV	10 ml	2 x 30 ml	3 x 85 ml
Binding Solution SBS	25 ml	120 ml	5 x 120 ml
Washing Solution A	30 ml	120 ml	3 x 200 ml
Washing Solution B2 (conc.)	10 ml (for 25 ml working solution)	50 ml (for 125 ml working solution)	3 x 80 ml (for 600 ml working solution)
Manual	1	1	1

6.2 Components not included in the kit

- 96 %–99.8 % Ethanol (molecular biology grade, undenaturated)

6.3 Related Products

- Deep Well Plate (96 square well, 2.0 ml 845-FX-8500025, 25 pcs)
- Deep Well Plate (96 square well, 2.0 ml 845-FX-8500115, 115 pcs)
- IPC16 Dummy Plate (sealed, 31-00258, 1 piece)

7 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-PPP-1116016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-PPP-1116096 845-PPP-1116480	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of absolute ethanol to **Washing Solution B2** and mix thoroughly. Always keep the bottle firmly closed!

845-PPP-1116016	Add 15 ml of **96-99.8 % ethanol** to 10 ml **Washing Solution B2 (conc.)**
845-PPP-1116096	Add 75 ml of **96-99.8 % ethanol** to 50 ml **Washing Solution B2 (conc.)**
845-PPP-1116480	Add 120 ml of **96-99.8 % ethanol** to 80 ml **Washing Solution B2 (conc.)**

8 Product specifications

1. Starting material:
   - fresh or frozen whole blood samples
   - stabilized with EDTA, citrate or heparin
   - 200 µl sample volume
   - 400 µl sample volume

2. Time for isolation:

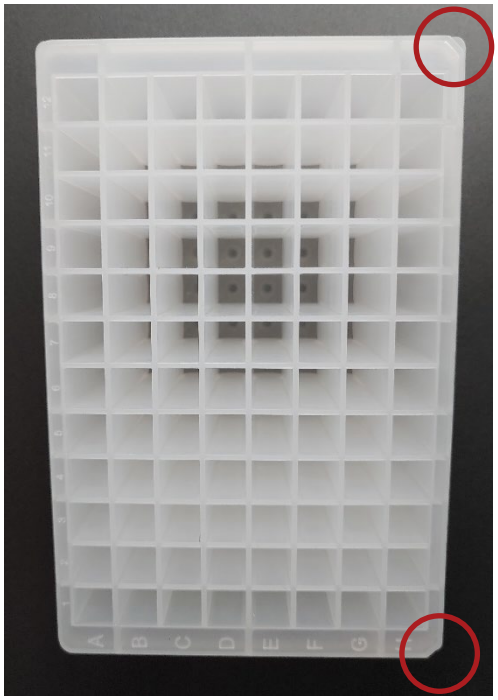
Extraction protocol InnuPureC16 / C16 touch	Protocol on InnuPureC16 / C16 touch	Time InnuPureC16 / C16 touch	Elution volumes
Int_Lysis_200_C16_04/ Internal Lysis 200 µl – 05	200 µl	79 / 77 min	20–500 µl
Int_Lysis_200_Fast_C16_04/ Internal Lysis 200 µl – Fast – 05	200 µl	59 / 58 min	20–500 µl
Int_Lysis_400_C16_04/ Internal Lysis 400 µl – 05	400 µl	90 / 89 min	20–500 µl

3. Typical yield:
   - Depending on sample quality
   - Depending amount of mononuclear blood cells
   - Average yield: 2–10 µg

9 Preparing the Reagent Plate for automated extraction

NOTE
The Deep Well Plates have to be filled manually prior to the automated extraction procedure.
Take care to fill the plates in the correct orientation: Engraved numbers do not coincide with row numbers quoted in the table below!

1. Place the Deep Well Plates in such a way, that the notched corners are facing to the right (see picture below).
2. In this orientation the upper row is row number 1.
3. Fill each cavity of one row with indicated volume of the corresponding solution as specified in the table (e.g. fill each of the eight cavities of row 1 with 940 µl of RNase-free water). Add **MAG Suspension F**, **sample** and **Proteinase K** as described in the chapter “Protocols for isolation of genomic DNA” on page 12.

Deep Well Plate	Row No.	Solution	Volume per cavity
	1	RNase-free Water	940 µl
	2	Lysis Solution CBV	500 µl
	3	empty	---
	4	empty	---
	5	empty	---
	6	Binding Solution SBS	1000 µl
	7	Washing Solution A	600 µl
	8	Washing Solution A	600 µl
	9	Washing Solution B2	600 µl
	10	Washing Solution B2	600 µl
	11	empty	---
	12	RNase-free Water	600 µl

10 Protocols for isolation of genomic DNA

10.1 Protocol 1: Isolation from 200 µl whole blood sample

NOTE
The lysis of the starting material is done automatically and is included in the InnuPure C16 / C16 *touch* extraction protocol.
It is important to mix the **MAG Suspension F** by vigorous shaking or vortexing before use (approx. 30 seconds).

1. Transfer **10 µl** of **MAG Suspension F** directly into the liquid of the **first cavity** of the Reagent Plate.
2. Add **200 µl whole blood sample** directly into the **third cavity** of the Reagent Plate.
3. Add **30 µl Proteinase K** to the **third cavity** of the Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 / C16 *touch*.
Please follow the instructions in chapter 12 on p. 12.

10.2 Protocol 2: Isolation from 400 µl whole blood sample

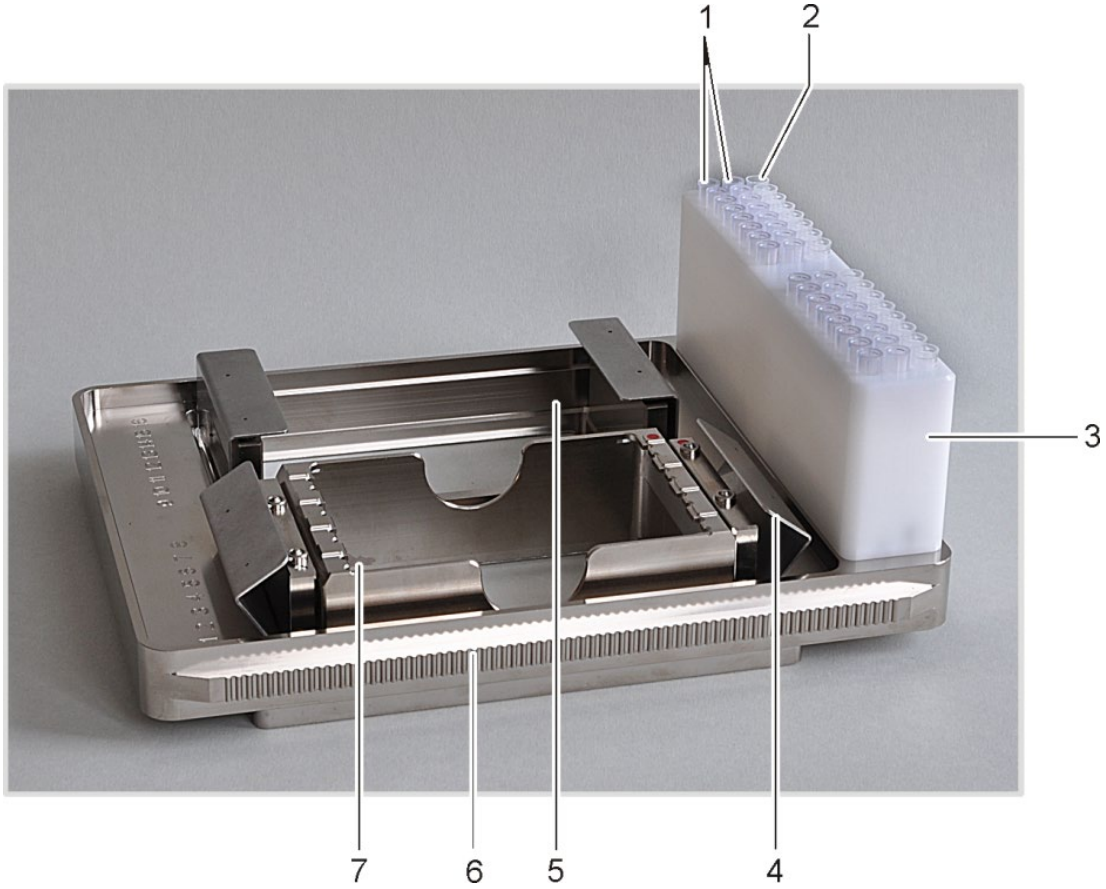
NOTE
The lysis of the starting material is done automatically and is included in the InnuPure C16 / C16 *touch* extraction protocol.
It is important to mix the **MAG Suspension F** by vigorous shaking or vortexing before use (approx. 30 seconds).

1. Transfer **10 µl** of **MAG Suspension F** directly into the liquid of the **first cavity** of the Reagent Plate.
2. Add **400 µl whole blood sample** directly into the **third cavity** of the Reagent Plate.
3. Add **50 µl Proteinase K** to the **third cavity** of the Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 / C16 *touch*.
Please follow the instructions in chapter 12 on p. 12.

11 Automated extraction using InnuPure C16 / C16 touch

11.1 Sample tray of InnuPure C16 / C16 touch



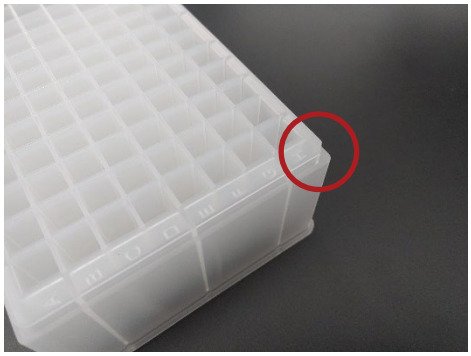
No. 1:	Filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Holding-down clamp
No. 5:	Sample block for Reagent Plates or adapter for Reagent Strips
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for Reagent Strips

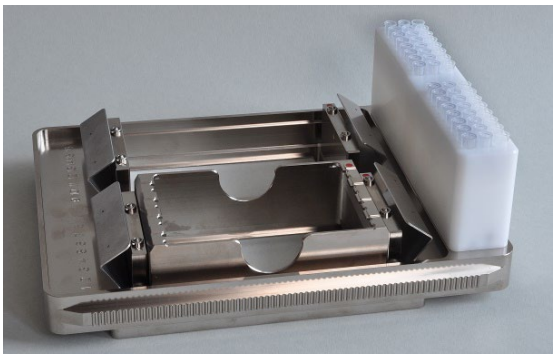
11.2 Preparing the sample tray of InnuPure C16 / C16 touch

1. Place the InnuPure C16 / C16 *touch* sample tray into the priming station and open the holding-down clamps of the sample tray!
2. Place the Reagent Plate into the holder of the sample tray. The notched corner of the Reagent Plate has to align with the colored dot on the holder.

Reagent Plate

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





CAUTION

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

3. Close the holding-down clamps to prevent the Reagent Plates from being pulled out of the holder during the extraction process.
4. For each extracted sample place two filter tips in the smaller holes of the tip block.
5. Place the Elution Tubes into the wider holes at the edge of the tip block. Empty sample positions do not need to be filled.

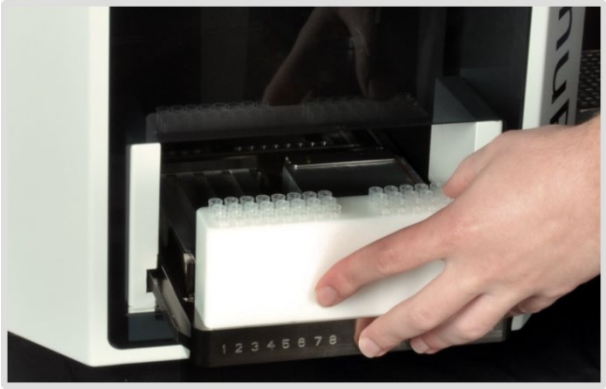
NOTE

Make sure that for every sample the tips and the elution vessel are in the corresponding positions in the tip block!

ATTENTION
It is possible to select between two different elution vessels! For smaller elution volumes up to 200 µl use Elution Strips (0.2 ml). For higher elution volumes up to 500 µl use Elution Tubes (0.65 ml) with corresponding Elution Caps (Stripes).

11.3 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 Plates forward into the sample tray adapter 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 injury
Immediately let go of the sample tray once it is being pulled in. Otherwise there is a risk of your hand being injured.

3. After pressing [Select Protocol] choose an appropriate extraction protocol on InnuPure C16 and press [Start]:

Extraction procedure	Protocol on InnuPureC16
Standard (maximum yield, approx. 55 minutes)	Ext_Lysis_200_C16_04
Fast (time-optimized, approx. 43 minutes)	Ext_Lysis_200_Fast_C16_04

4. Enter the recommended **elution volume** of **200 µl** and press [OK].

NOTE
It is possible to adjust the elution volume values from 20 µl up to 500 µl.

5. If needed, choose log-file and enter sample IDs, press [OK] or [CANCEL].

NOTE
It is possible to enter sample IDs and to create a run logfile. Find detailed information how to start an extraction protocol using InnuPure C16 in the user manual(→ "6.3.5 Using the sample setup tool", p. 37)

6. After completion of the protocol press [NEXT] and the sample tray will be moved out of the device.

NOTE
The chosen protocol is performed by the device. After the protocol is finished, the tray with the purified samples will be moved out of the device upon pressing [NEXT]. The message "Program finished" will be displayed on the screen of the device!

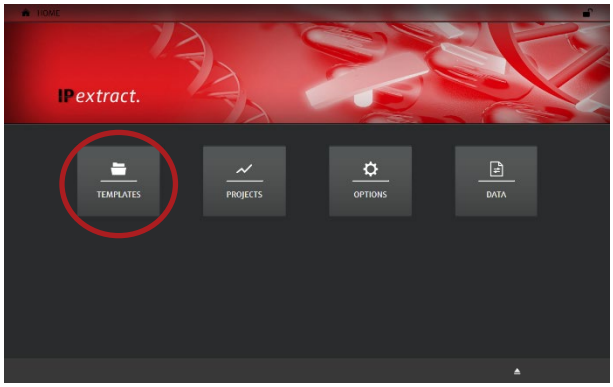
7. Remove the sample tray from the adapter of the InnuPure C16 and place it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

11.4 Starting the InnuPure C16 touch

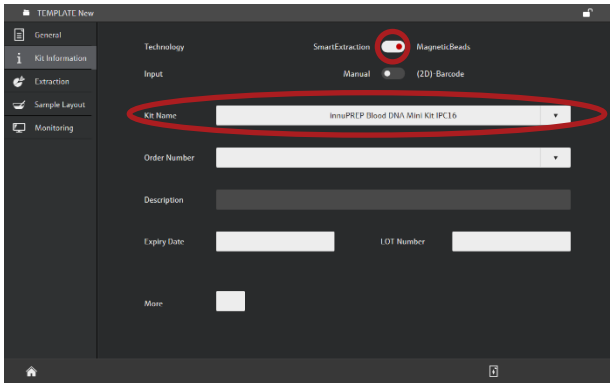
NOTE
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

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 “MagneticBeads”!
5. Choose your desired kit from the drop-down list “Kit Name”!

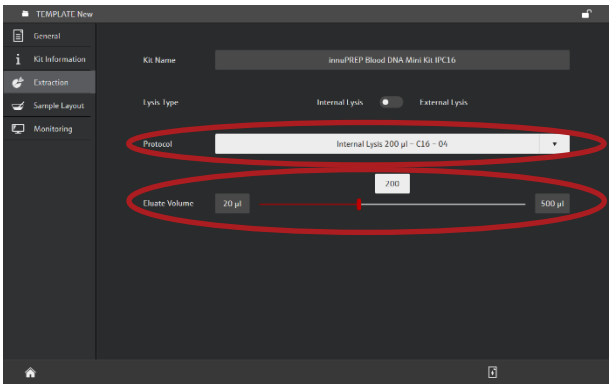


NOTE
“Kit Information” tab

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

Extraction procedure	Protocol on InnuPureC16 touch
Protocol 1 (Starting volume: 200 µl) Elution volumes 20–500 µl	Internal Lysis 200 µl – 05 Internal Lysis 200 µl – Fast – 05
Protocol 2 (Starting volume: 400 µl) Elution volumes 20–500 µl	Internal Lysis 400 µl – 05

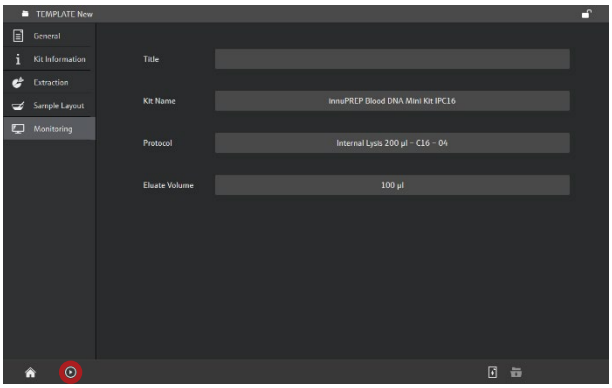
8. Adjust the “Eluate Volume“ using the slider or the text field.



NOTE
“Extraction” tab

The recommended elution volume is 200 µl.

9. Choose the tab “Monitoring” and start the protocol by tapping the start button.



NOTE
“Monitoring” tab

10. Follow the instructions displayed on the tablet screen.

11. After loading the tray into the device, a message appears reminding you that all cavities must be open before starting. If you have closed the Reagent Plates with a foil, please remove it. Please ignore the message if you have not sealed the Reagent Plates. The message must still be confirmed for the protocol to start.

12. 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.

13. The Elution Tubes contain the extracted DNA. Close the lids and store the DNA under adequate conditions.

NOTE
Store DNA 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 genomic DNA	
No extracted DNA	No magnetic beads added to cavity 1. Please add 10 µl **MAG Suspension F** to **cavity 1** prior the extraction procedure. Ensure **Mag Suspension F** has been mixed well before use.
Content of nucleic acid in sample is insufficient.	Use more starting material, e.g. use 400 µl instead of 200 µl sample. Ensure that the appropriate extraction protocol is chosen.
Insufficient lysis of starting material.	Ensure to use the required volume of **Proteinase K** for current protocols, e.g. 30 µl **Proteinase K** for 200 µl of sample, but 50 µl **Proteinase K** for 400 µl of sample.
Elution volume too high.	Decrease the elution volume. The suggested elution volume is 200 µl. Please note that lowering the elution volume will not increase the yield proportionally!
Inadequate extraction.	Presence of inhibiting substances in the starting material. Please use the kit only for samples that match the requirements declared in “Product specifications”. Use internal controls for verification of the extraction procedure.

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