

# Instructions for Use

## Life Science Kits & Assays



**innuPREP Blood DNA Mini Kit - IPC16**

**Order No.:**

845-IPS-1116016	16 reactions
845-IPP-1116016	16 reactions
845-IPS-1116096	96 reactions
845-IPP-1116096	96 reactions
845-IPP-1116480	480 reactions

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Made in Germany!	info.innu@ist-ag.com

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# 1 Introduction

## 1.1 Intended use

The innuPREP Blood DNA Mini Kit – IPC16 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 *touch*. The MAG Suspension F and the whole blood sample are transferred without any external handling steps into the Reagent Strips or Reagent Plate of the kit, which is already prefilled with all extraction reagents needed for the extraction process. After addition of Proteinase K, the extraction procedure begins with sample lysis, followed by binding of DNA to surface-modified magnetic particles. After washing steps the DNA is eluted from the magnetic particles with RNase-free water and is now ready to use for downstream applications. The extraction chemistry in combination with the InnuPure C16 *touch* protocol are optimized to get maximum of yield and quality.

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### CONSULT INSTRUCTION FOR USE


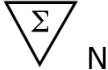
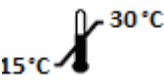







This package insert must be read carefully before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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## 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
	<b>REF</b> Catalogue number.
	<b>Content</b> Contains sufficient reagents for <N> tests.
	<b>Storage conditions</b> Store at room temperature, unless otherwise specified.
	<b>Consult instructions for use</b> This information must be observed to avoid improper use of the kit and the kit components.
	<b>Expiry date</b>
	<b>Lot number</b> The number of the kit charge.
	<b>Manufactured by</b> Contact information of manufacturer.
	<b>For single use only</b> Do not use components for a second time.
	<b>Note / Attention</b> 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

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### 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, which are shown.

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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.

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### FOR SINGLE USE ONLY!

This kit is made for single use only!

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### ATTENTION!

Don't eat or drink components of the kit!

The kit shall only be handled by educated personnel in a laboratory environment!

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If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. Analytik Jena AG has not tested the liquid waste generated during 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 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.

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### ATTENTION!

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

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**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.

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

### 3 Storage conditions

The kit is shipped at ambient temperature.

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

All other components of the innuPREP Blood DNA Mini Kit – IPC16 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 **innuPREP Blood DNA Mini Kit – IPC16** or other IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information in Germany please contact [info.inn@ist-ag.com](mailto:info.inn@ist-ag.com). 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. 2) (→ "Product specifications" p. 9). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalent regulations required in other countries.

All products sold by 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.

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### NOTE

The kit is for research use only!

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## 6 Performance and Product use limitations

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.

The kit is designed to avoid cross-contamination between samples and allows safe handling of potentially infectious samples. Genomic DNA is eluted in RNase-free water and is ready to use for different downstream applications or storage at -22 °C to -18 °C. For long-term storage -80 °C is recommended.

The kit is not intended for use with serum, plasma, tissue, bone marrow and not for the isolation and purification of bacterial, fungal or parasite nucleic acids. The performance of the kit in isolating and purifying genomic nucleic acids from other body fluids, such as urine and cerebrospinal fluid, has not been evaluated. When changing the starting material no guarantee in operability is issued.

### 6.1 Specificity

The specificity of detection of genomic DNA depends on the detection system and detection device.

### 6.2 Sensitivity




The sensitivity of detection of genomic DNA depends on the amount of DNA in the starting sample volume, on the volume of sample and of inhibitors in the human whole blood sample corresponding with the detection system.

The amount of isolated genomic DNA depends on the sample type (EDTA, heparin or citrate stabilized), sample transport, storage and age.

As yield may vary, quantitative real-time PCR is recommended for determination of DNA concentration.

## 7 Kit components

### 7.1 Components included in the kit

	 16	 96	 480
<b>REF</b>	845-IPS-1116016 <sup>a</sup> 845-IPP-1116016 <sup>b</sup>	845-IPS-1116096 <sup>a</sup> 845-IPP-1116096 <sup>b</sup>	845-IPP-1116480 <sup>b</sup>
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
Reagent Strip J <sup>a</sup>	16 (pre-filled, sealed)	96 (pre-filled, sealed)	--
Reagent Plate J <sup>b</sup>	2 (pre-filled, sealed)	12 (pre-filled, sealed)	60 (pre-filled, sealed)
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
Manual	1	1	1

### 7.2 Components not included in the kit

- ddH<sub>2</sub>O for dissolving Proteinase K
- Piercing Tool (12 well Piercer; 845-PTS-0000005, IST Innuscreen GmbH, Germany)

## 8 Initial steps before starting

- 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
- Add the indicated amount of ddH<sub>2</sub>O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-IPS-1116016	Add 0.3 ml ddH <sub>2</sub> O to lyophilized Proteinase K.
845-IPP-1116016	
845-IPS-1116096	Add 1.5 ml ddH <sub>2</sub> O to lyophilized Proteinase K.
845-IPP-1116096	
845-IPP-1116480	

## 9 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:

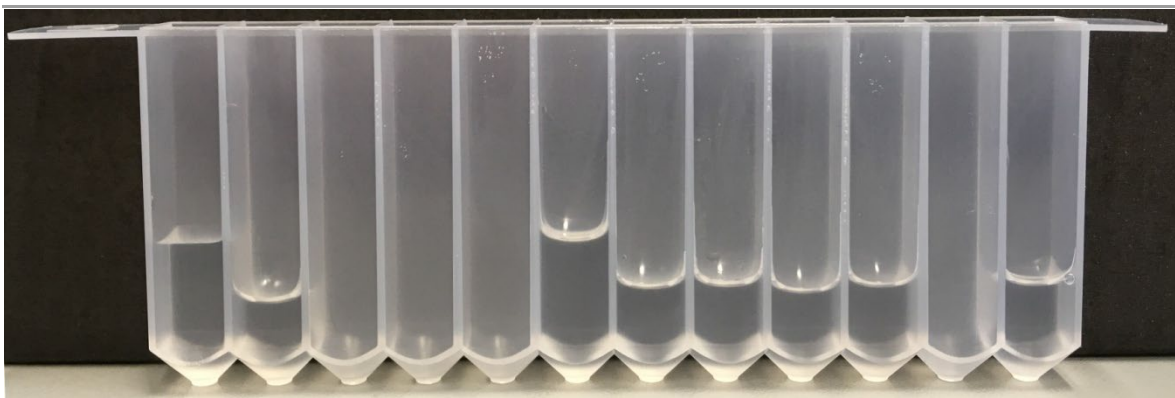
Extraction protocol InnuPure C16 <i>touch</i>	Protocol on InnuPure C16 <i>touch</i>	Time InnuPure C16 <i>touch</i>	Elution volumes
Internal Lysis 200 µl – 05	200 µl	77 min	20–500 µl
Internal Lysis 200 µl – Fast – 05	200 µl	58 min	20–500 µl
Internal Lysis 400 µl – 05	400 µl	89 min	20–500 µl

3. Typical yield:

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

# 10    Preparing Reagent Plate / Strip for automated extraction

## 10.1    General filling scheme of reagent reservoir



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

### 10.2 Unpacking of Reagent Plate and piercing of sealing foil

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#### NOTE

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

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Reagent Plates or Reagent Strips are delivered wrapped into plastic bags for transport protection.

Carefully open the overpack of Reagent Plates by using scissors.

### 10.3 Piercing of sealing foil of Reagent Plate or Reagent Strip

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#### NOTE

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

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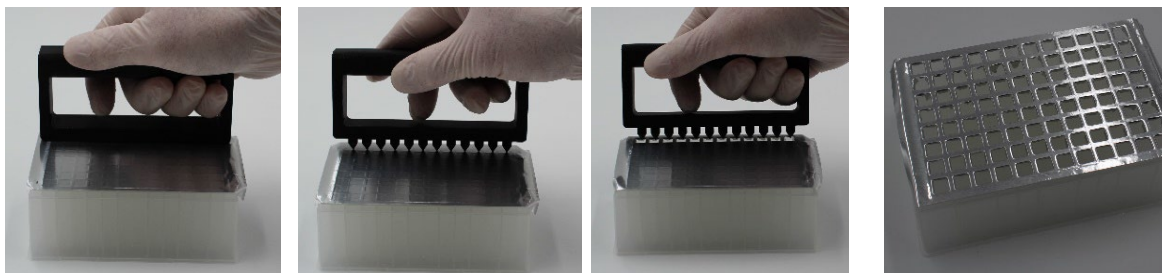
Reagent Plates or Strips are prefilled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (12 well piercer).

Keep the Reagent Plates or Strips in a horizontal position to avoid spilling of the reagents while piercing of the foil. Open all cavities (one row per sample).

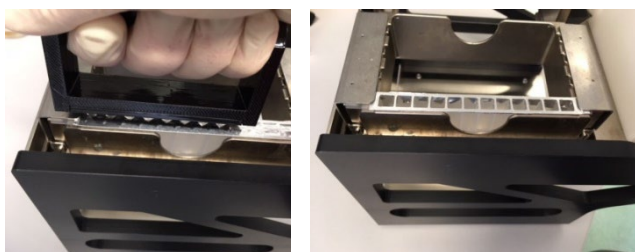
## Preparing Reagent Plate / Strip for automated extraction

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### Using Reagent Plates



### Using Reagent Strips



## 11 Protocols for isolation of genomic DNA

### 11.1 Protocol 1: Isolation from 200 µl whole blood sample

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#### NOTE

The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.

It is important to mix the **MAG Suspension F** by vigorous shaking or vortexing before use (approx. 30 seconds).

Ensure the foils of Reagent Plate or Reagent Strips have been pierced (→ „Preparing Reagent Plate / Strip for automated extraction“ p. 10).

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

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#### NOTE

The sample will be processed using the InnuPure C16 *touch*. Please follow the instruction of chapter 12 p. 15.

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## 11.2 Protocol 2: Isolation from 400 µl whole blood sample

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### NOTE

The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.

It is important to mix the **MAG Suspension F** by vigorous shaking or vortexing before use (approx. 30 seconds).

Ensure the foils of Reagent Plate or Reagent Strips have been pierced (→ „Preparing Reagent Plate / Strip for automated extraction“ p. 10).

---

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

### NOTE

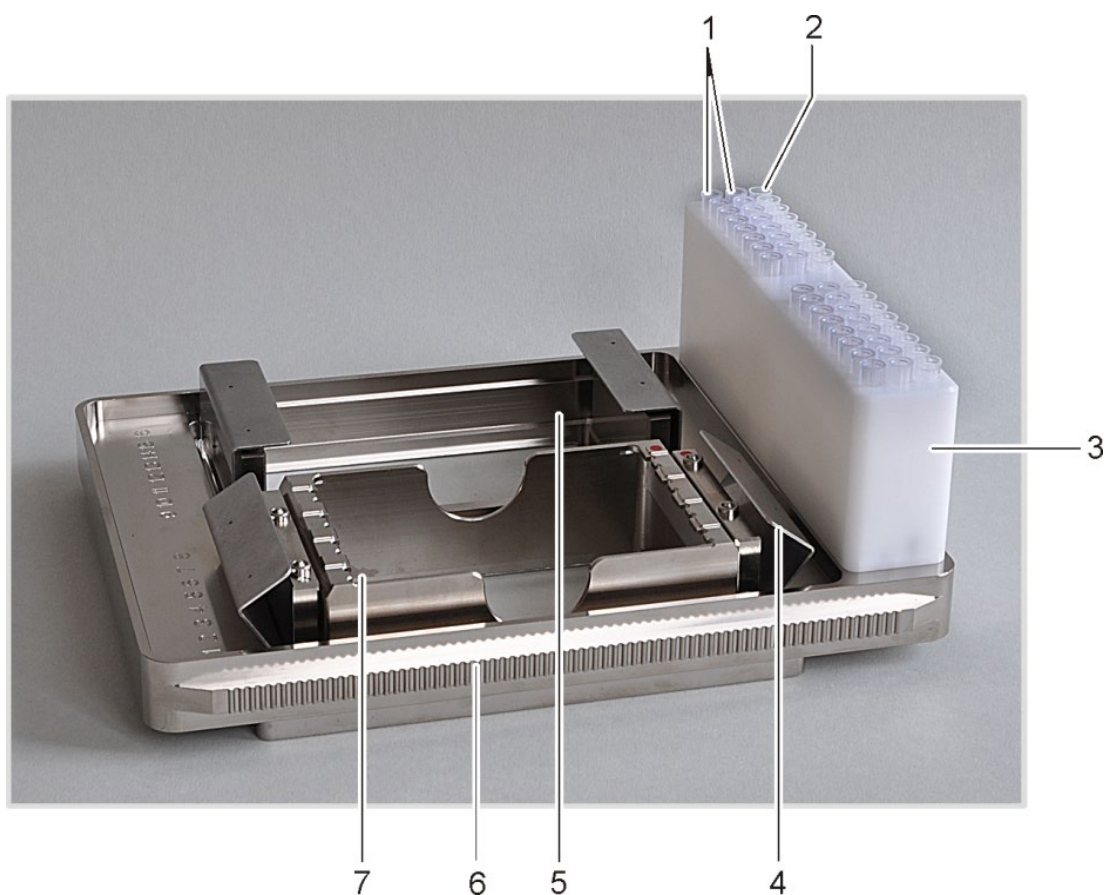
The sample will be processed using the InnuPure C16 *touch*. Please follow the instruction of chapter 12 p. 15.

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## 12 Automated extraction using InnuPure C16 touch

### 12.1 Sample tray of InnuPure 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:** Guide rail

**No. 7:** Adapter for reagent strips

### 12.2 Preparing sample tray of InnuPure C16 touch

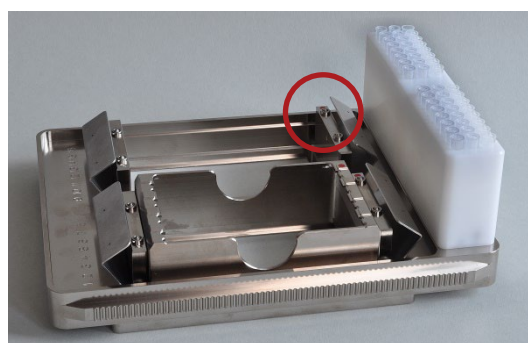
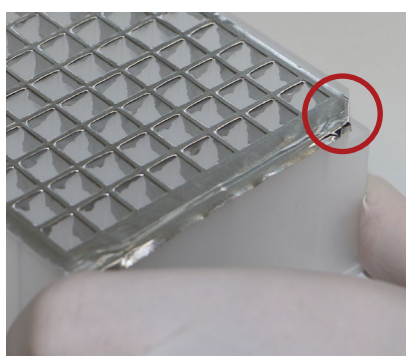
#### NOTE

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

1. Place the InnuPure 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 with Reagent Strips into the holder of the sample tray. Using Reagent Plates, the notched corner of the Reagent Plate must align with the colored dot at the holder. Using adapters and Reagent Strips, the colored dot of the adapter must 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.

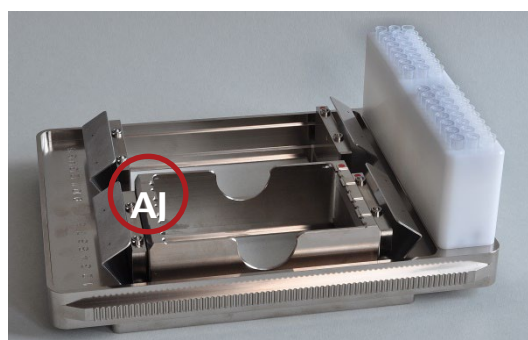
#### Reagent Plate

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



#### Reagent Strips

Place the 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.



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**CAUTION**

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

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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 two filter tips in the smaller drill holes of the tip block.
  5. Place the Elution Tubes into the wider drill holes 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 strip the tips and the elution vessel are in the corresponding positions in the tip block!

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**IMPORTANT NOTE**

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).

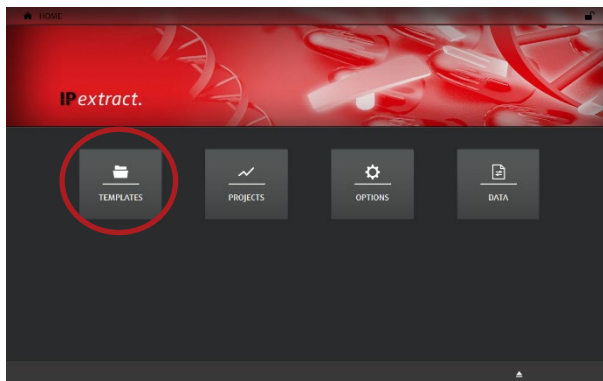
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### 12.3 Starting the InnuPure C16 *touch*

#### 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*.

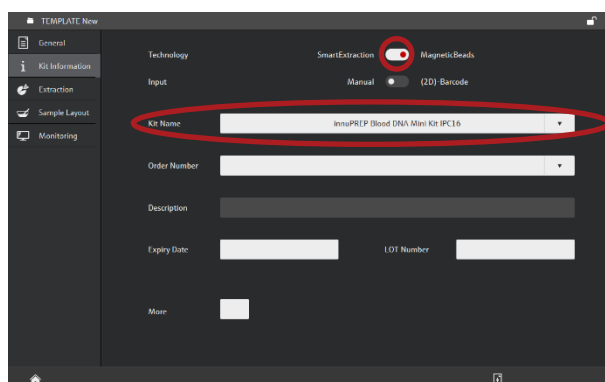
1. Switch on the InnuPure C16 *touch* and the tablet computer. Wait until the home screen of IPextract is displayed on the tablet screen.



#### NOTE

Home screen of IPextract

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 "Kit Name"!



#### NOTE

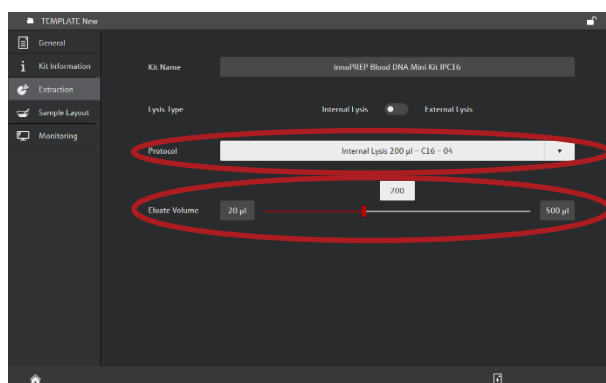
"Kit Information" tab

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
<b>Protocol 1</b> (Starting volume: 200 µl) Elution volumes 20–500 µl	Internal Lysis 200 µl – 05 Internal Lysis 200 µl – Fast – 05
<b>Protocol 2</b> (Starting volume: 400 µl) Elution volumes 20–500 µl	Internal Lysis 400 µl – 05

## 8. Adjust your desired "Eluate Volume" using the slider or the text field.

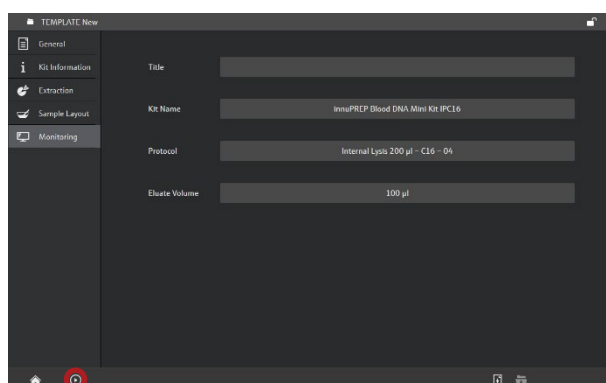


### NOTE

"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. 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 adequate conditions.

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### **NOTE**

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

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## 13 Troubleshooting

Problem / probable cause	Comments and suggestions
<b>Low amount of extracted genomic DNA</b>	
No extracted DNA	<p>No magnetic beads added to cavity 1. Please add 10 µl <b>MAG Suspension F</b> to <b>cavity 1</b> prior the extraction procedure.</p> <p>Ensure <b>Mag Suspension F</b> has mixed well before use.</p>
Content of nucleic acid in sample insufficient.	<p>Use more starting material, e.g. use 400 µl instead of 200 µl sample. Ensure to choose the appropriate extraction protocol.</p>
Insufficient lysis of starting material.	<p>Ensure to use the required volume of <b>Proteinase K</b> for current protocols, e.g. 30 µl <b>Proteinase K</b> for 200 µl of sample, but 50 µl <b>Proteinase K</b> for 400 µl of sample.</p>
Elution volume too high.	<p>Decrease the elution volume. The suggested elution volume is 200 µl. Please note that lowering the elution volume will not necessarily increase the yield proportional!</p>
Inadequate extraction.	<p>Inhibiting substances in starting material. Please use the kit only for samples that match the requirements declared in "Product specifications".</p> <p>Use internal controls for verification of extraction procedure.</p>

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