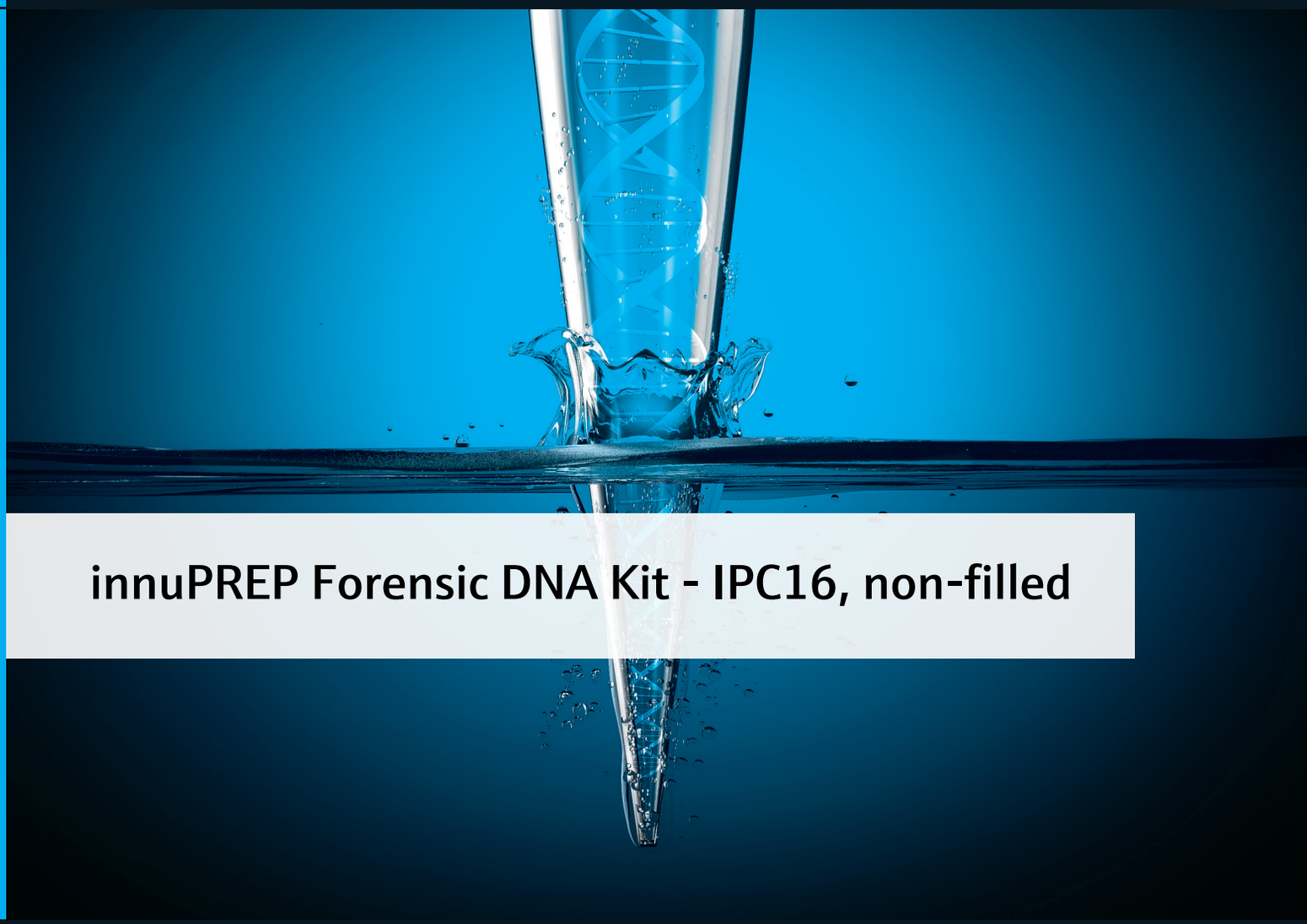
Instructions for Use
Life Science Kits & Assays



innuPREP Forensic DNA Kit - IPC16, non-filled

Innuscreen GmbH

Order No.:

845-PPP-2416016 16 reactions
845-PPP-2416096 96 reactions
845-PPP-2416480 480 reactions

Publication No.: HB_PPP_e-2416_e_220927

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

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Contents

1 Introduction

1.1 Intended use

The **innuPREP Forensic DNA Kit - IPC16, non-filled** has been designed for the automated isolation of DNA from small amounts of different types of forensic samples like hair or hair roots, stains of blood, saliva or sperm, fingernails, cigarette butts, bubble gum, buccal swabs, stamps and envelopes as well as fingerprints on different surfaces. The extraction procedure is based on a new-patented chemistry.

The extraction procedure starts with an external lysis step. After the external lysis step the sample is transferred into the Reagent Plate of the kit, which must be prefilled with all reagents needed for the extraction process. The extraction procedure runs automatically on the InnuPure C16 / C16 *touch*. The extraction process is based on binding of DNA on surface modified magnetic particles. After several washing steps the nucleic acids are eluted from the magnetic particles and are 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.

Furthermore, the kit contains a Carrier Mix with a Carrier RNA as well as Internal Control DNA (IC DNA) and RNA (IC RNA) for better recovery of minute amounts of sample DNA as well as verification of the successful extraction process. The IC DNA can be detected by real-time PCR with a corresponding real-time PCR detection kit.

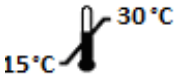
Please note that the eluates of the kit contain both, sample DNA and Carrier Mix. Therefore, it is not possible to quantify the isolated nucleic acids by photometric or fluorometric methods when using the Carrier Mix. Thus, other methods for quantification such as specific quantitative PCR or real-time PCR systems are recommended. Furthermore, Carrier RNA may inhibit PCR reactions. The amount of added Carrier RNA must therefore be carefully optimized depending on the individual PCR system used.

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** Catalogue number.
	**Content** Contains sufficient reagents for <N> tests.
	**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 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** and dissolved and lyophilized **Proteinase K** at 4 °C to 8 °C.

Store lyophilized and dissolved **Carrier Mix** at -22 °C to -18 °C. Aliquot dissolved **Carrier Mix** and do not freeze and thaw it more than 3 times!

All other components of the **innuPREP Forensic DNA 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 product has been 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 Forensic DNA 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 components included in the kit

	Σ 16	Σ 96	Σ 480
REF	845-PPP-2416016	845-PPP-2416096	845-PPP-2416480
MAG Suspension	1.5 ml	5.5 ml	3 x 9 ml
Proteinase K	For 2 × 0.3 ml working solution	For 2 × 1.5 ml working solution	For 7 × 1.5 ml working solution
Carrier Mix	For 1 × 1.25 ml working solution	For 1 × 1.25 ml working solution	For 5 × 1.25 ml working solution
Lysis Solution CBV	10 ml	25 ml	125 ml
Binding Solution SBS	25 ml	120 ml	2 x 250 ml
Washing Solution A	30 ml	120 ml	600 ml
Washing Solution B2 (conc.)	10 ml (for 25 ml working solution)	50 ml (for 125 ml working solution)	240 ml (for 600 ml working solution)
RNase-free Water	30 ml	2 x 80 ml	4 x 200 ml
Deep Well Plate (2.0 ml)	2	12	60
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 Stripes	2	12	5 × 12
Manual	1	1	1

6.2 Components not included in the kit

- 1.5 ml tubes and 2.0 ml tubes, optional
- ddH_2O / RNase-free Water for dissolving **Proteinase K** / **Carrier Mix** and for all protocols
- 1 M DTT solution for Protocols 2 and 3
- 96 %–99.8 % Ethanol (molecular biology grade, undenatured)

6.3 Related Products

- Deep Well Plate (96 square well, 2.0 ml 845-FX-8500025, 25 pcs)

7 Initial steps before starting

- Add the indicated amount of ddH_2O to **Proteinase K**, mix thoroughly and store as described above.

845-PPP-2416016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-PPP-2416066 845-PPP-2416480	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-2416016	Add 15 ml **ethanol** to 10 ml **Washing Solution B2 (conc.).**
845-PPP-2416096	Add 75 ml **ethanol** to 50 ml **Washing Solution B2 (conc.).**
845-PPP-2416480	Add 360 ml **ethanol** to 80 ml **Washing Solution B2 (conc.).**

- Add the indicated amount of RNase-free Water to **Carrier Mix**, mix thoroughly and store as described above.

845-PPP-2416016 845-PPP-2416096 845-PPP-2416480	Add **1.25 ml RNase-free Water** to lyophilized **Carrier Mix.**

- Pre-heat thermal mixer or water bath to 50 °C.

- Centrifugation steps should be carried out at room temperature.
- Prepare mixture of **Lysis Solution CBV / Carrier Mix** according to the table below and store the mixture at 4–8 °C for a maximum of 7 days.

Component	16 samples	96 samples	n samples
Lysis Solution CBV	4 ml	24 ml	250 µl x n samples
Carrier Mix	200 µl	1.2 ml	12.5 µl x n samples
Final volume	4.2 ml	25.2 ml	262.5 µl x n samples

8 Product specifications

1. Starting material:

- Swabs from different surfaces (e.g. cups, bottles, fingerprints)
- Blood samples
- Sperm samples
- Hair, hair roots or barb hairs
- Envelopes
- Fingernails
- Cigarette butts or paper
- Chewing gum

2. Time for isolation:

External lysis step: approx. 1–2 hours

Extraction protocol	Protocol on InnuPureC16 / C16 touch	Time InnuPureC16 / C16 touch	Elution volumes
Ext_Lysis_200_C16_04/ External Lysis 200µl – 05	200 µl	55 / 52 min	20–500 µl
Ext_Lysis_200_Fast_C16_04/	200 µl	43 / 41 min	20–500 µl

External Lysis 200µl – Fast – 05

3. Typical yield:

Not determined. The yield depends on the type and amount of starting material

9 Usage of Carrier Mix

In addition to carrier RNA, the **Carrier Mix** contains internal control DNA and RNA (IC DNA and IC RNA). Both can be detected by real-time PCR using the following assay.

Name	Amount	Order No.
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

If customized extraction controls are used, please add these components to the mixture of **Lysis Solution CBV** and **Carrier Mix.**

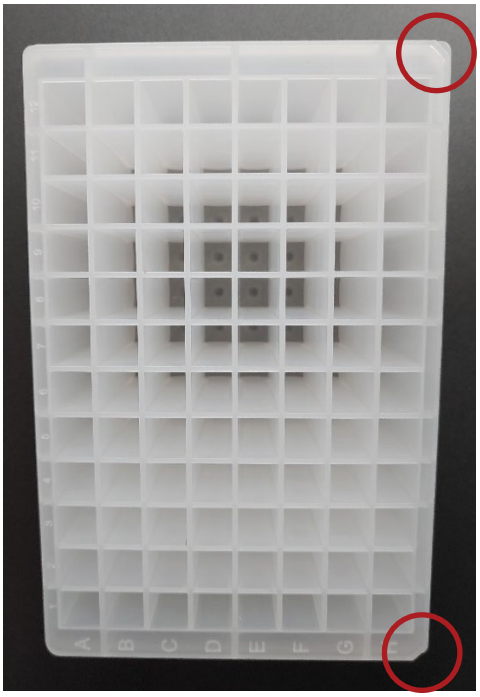
10 Preparing the Reagent Plate for automated extraction

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 900 µl of RNase-free water). Add **MAG Suspension** and the lysed **sample** as described in chapter 12 p. 18.

Deep Well Plate	Row No.	Solution	Volume per cavity
	1	RNase-free Water	900 µl
	2	empty	---
	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

11 Protocols for isolation of DNA

11.1 Protocol 1: Isolation from buccal swab samples from different surfaces (cups, bottles, fingerprints etc.)

NOTE
To get maximum yield of DNA it is essential to leave the swab in the 1.5 ml tube for the entire time of lysis. It is possible to cut the shaft of the swab, so that you can close the cap of the tube. The removal of the swab from the tube ahead of time will lead to a dramatically reduced final yield!

1. Place the swab into a 1.5 ml tube and add:

   200 µl ddH_2O,

   200 µl Lysis Solution CBV / Carrier Mix (→ "Initial steps before starting", p. 8) and

   20 µl Proteinase K

   Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 50 °C for 15 minutes.

NOTE
Assure that the swab is immersed in the Lysis Solution throughout the lysis!

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. Absence of shaking will reduce lysis efficiency!

2. After lysis, remove the swab from the 1.5 ml tube and squeeze it against the wall of the tube to remove all Lysis Solution CBV.
3. Proceed with automated extraction (→ "Loading the sample into the Reagent Plate", p.15).

11.2 Protocol 2: Extraction from sperm samples, hair roots, barb hairs, fingernails

1. Cut the material into small pieces and transfer them into a 1.5 ml reaction tube and add:

   200 µl ddH_2O,

   200 µl Lysis Solution CBV / Carrier Mix (→ "Initial steps before starting", p. 8),

   20 µl Proteinase K and

   30 µl DTT solution (1 M) (not provided)

   Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 50 °C for at least 2 hours.

NOTE
Assure that the sample is immersed in the Lysis Solution throughout the lysis!

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. Absence of shaking will reduce lysis efficiency!

2. Centrifuge the 1.5 ml tube at 10,000 x g (12,000 rpm) for 1 minute to spin down unlysed material. Use the supernatant for the subsequent extraction process.

3. Proceed with automated extraction (→ "Loading the sample into the Reagent Plate", p.15).

11.3 Protocol 3: Extraction from blood samples, envelopes, cigarette butts or paper and chewing gum

1. Cut the material into small pieces and transfer it into a 1.5 ml reaction tube and add:

   200 µl ddH_2O,

   200 µl Lysis Solution CBV / Carrier Mix (→ "Initial steps before starting", p. 8),

   20 µl Proteinase K and

   30 µl DTT solution (1 M) (not provided)

   Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 50 °C for at least 2 hours.

NOTE
Assure that the sample is immersed in the Lysis Solution throughout the lysis!

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. Absence of shaking will reduce lysis efficiency!

2. Centrifuge the 1.5 ml tube at 10,000 x g (12,000 rpm) for 1 minute to spin down unlysed material. Use the supernatant for the subsequent extraction process.
3. Proceed with automated extraction (→ "Loading the sample into the Reagent Plate", p.15).

12 Loading the sample into the Reagent Plate

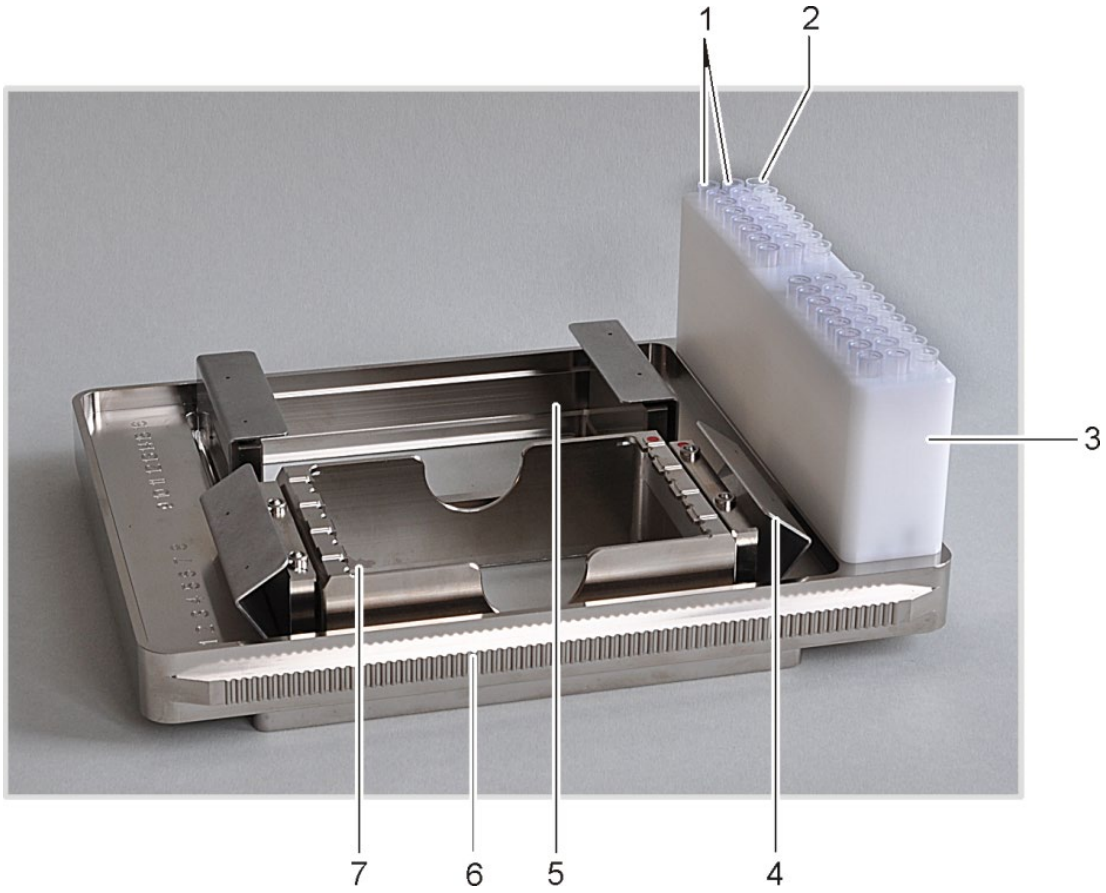
NOTE
It is important to mix the **MAG Suspension** by vigorous shaking or vortexing before use (approx. 30 seconds)!

1. Transfer **50 µl** of **MAG Suspension** directly into the liquid of the **first cavity** of the Reagent Plate.
2. Transfer **400 µl** of the **lysed sample** into the **third cavity** of the Reagent Plate. Avoid carry-over of solid material!

NOTE
The sample will be processed using the InnuPure C16 / C16 *touch*.
Please follow the instructions of chapter 13 p. 16.

13 Automated extraction using InnuPure C16 / C16 touch

13.1 Sample tray of InnuPure C16 / C16 touch



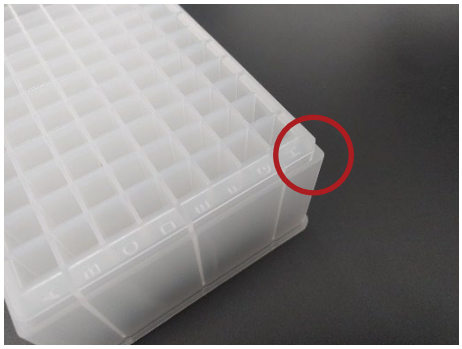
No. 1:	Filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Holding-down clamp
No. 5:	Sample block for Reagent Plates or adapter for Reagent Strips
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for Reagent Strips

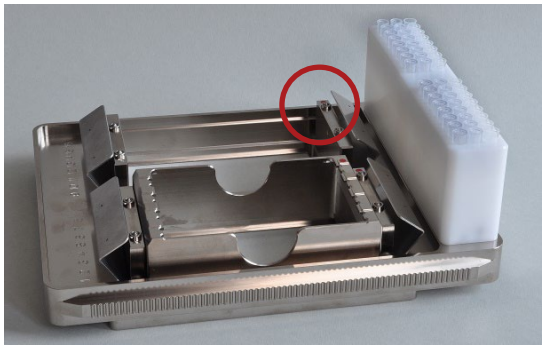
13.2 Preparing the sample tray of InnuPure C16 / C16 touch

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.

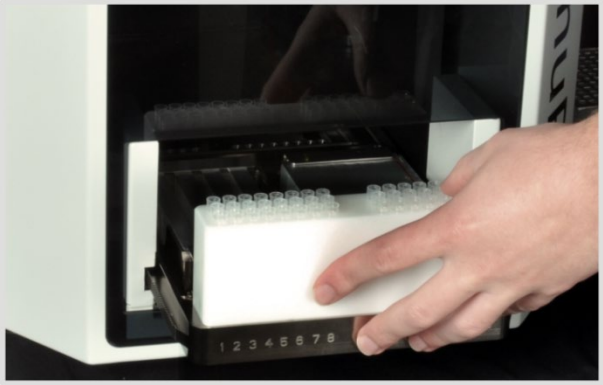
NOTE

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 small elution volumes up to 200 µl use Elution Stripes (0.2 ml). For high elution volumes up to 500 µl use Elution Tubes (0.65 ml) with corresponding Elution Caps (Stripes).

13.3 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 **100 µl** and press [OK].

NOTE
It is possible to adjust the elution volume values from20 µl 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 on 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]. 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!

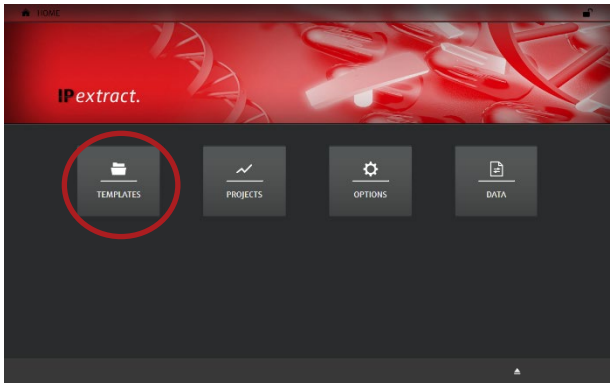
7. Remove the sample tray from the adapter of the InnuPure C16 and place it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

13.4 Starting the InnuPureC16 touch

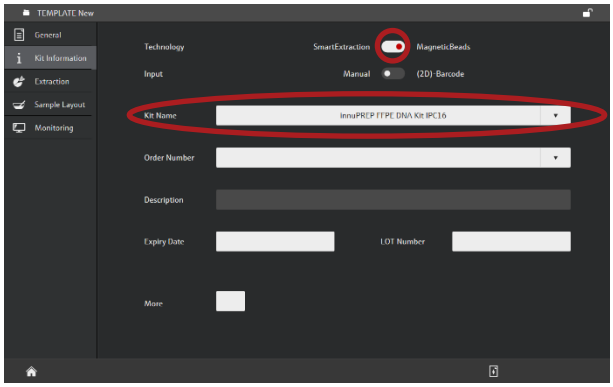
NOTE
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

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”!

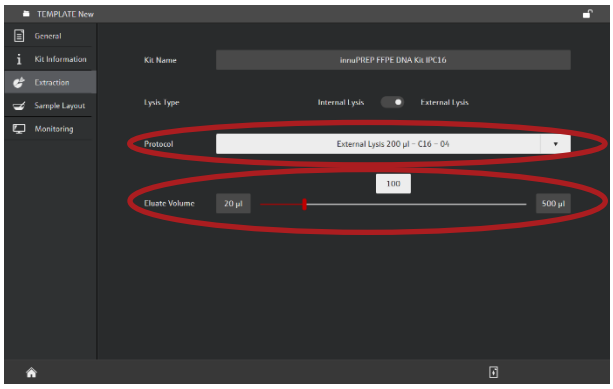


NOTE
“Kit Information” tab

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

Extraction procedure	Protocol on InnuPure C16 touch
Standard (maximum yield, approx. 52 minutes)	External Lysis 200 µl - 05
Fast (time-optimized, approx. 41 minutes)	External Lysis 200 µl - Fast - 05

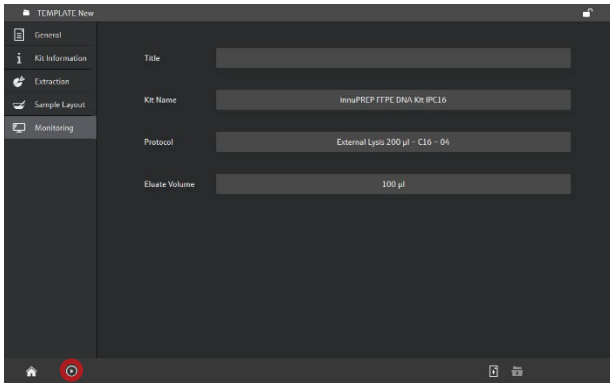
8. Adjust the “Eluate Volume“ using the slider or the text field.



NOTE
“Extraction” tab

The recommended elution volume is 100 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 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 proper conditions.

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

14 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 **50 µl MAG Suspension to cavity 1** prior the extraction procedure. Ensure **MAG Suspension** was mixed well before use.
No extracted DNA	Ensure that the **Proteinase K** and **Carrier Mix** have been prepared according to the instructions. Ensure that the **Carrier Mix** and **Lysis Solution CBV / Carrier Mix** have been prepared according to the instructions.
Poor quality of extracted DNA	Avoid carry-over of residual sample material when transferring lysed sample to cavity 3 of the Reagent Plate.
Insufficient lysis of starting material	Perform lysis at 50 °C. Ensure that the required volume of **Lysis Solution CBV / Carrier Mix mixture is used.**
Elution volume too high	Decrease the elution volume. The suggested elution volume is 100 µl. Please note that lowering the elution volume will not increase the yield proportionally!
Downstream application insufficient	Carrier RNA may inhibit PCR reactions. Thus, the amount of added Carrier RNA may thus be carefully optimized depending on the individual PCR system used.

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