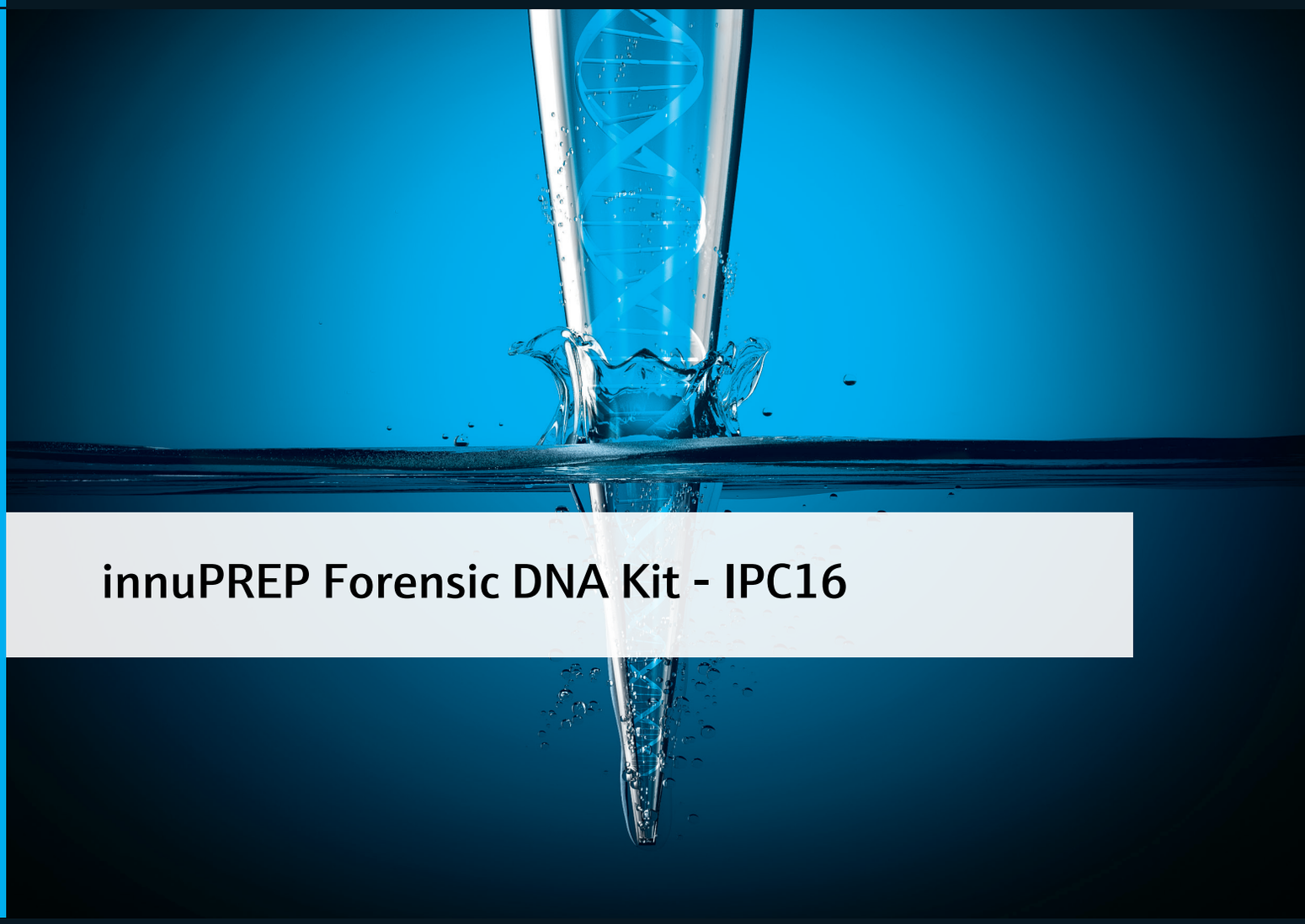
Instructions for Use

Life Science Kits & Assays



innuPREP Forensic DNA Kit - IPC16

Innuscreen GmbH

Order No.:

845-IPS-2416016	16 reactions
845-IPP-2416016	16 reactions
845-IPS-2416096	96 reactions
845-IPP-2416096	96 reactions
845-IPP-2416480	480 reactions

Publication No.: HB_IP-2416_e_220328

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 has been designed for the automated isolation of DNA from small amounts of different types of forensic samples like hairs or hair roots, stains of blood, saliva or sperm, finger nails, 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 Strip or Reagent Plate of the kit, which is already prefilled with all extraction reagents needed for the extraction process (except MAG Suspension). The following extraction process runs automatically on the InnuPure C16 / C16 *touch*. The extraction process is based on binding of the DNA on surface modified magnetic particles. After washing steps the nucleic acid is eluted from the magnetic particles and is now ready to use. The extraction chemistry in combination with the InnuPure C16 / C16 *touch* protocol are optimized to get maximum of yield and quality.

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

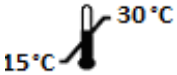
Please note that the eluates of the kit contain both sample DNA and Carrier Mix. Therefore, it is not possible to quantify the isolated nucleic acids by photometric or fluorometric methods when using the Carrier Mix. Thus other methods for quantification such as specific quantitative PCR or real-time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

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** 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” p. 5).
- 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, 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. IST Innuscreen GmbH 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.

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 **Proteinase K** and **MAG Suspension** 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 should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

For further information see chapter ”Kit components“ p. 9.

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 Forensic DNA Kit – IPC16 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 (→ “Intended use” p. 3) (→ “Product specifications” p. 11). 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.

6 Kit components

6.1 Included kit components

	Σ 16	Σ 96	Σ 480
REF	845-IPS-2416016[a] 845-IPP-2416016[b]	845-IPS-2416096[a] 845-IPP-2416096[b]	845-IPP-2416480[b]
MAG Suspension	1.5 ml	5.5 ml	3 x 9 ml
Lysis Solution CBV	10 ml	25 ml	125 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
RNase-free Water	1 × 2 ml	1 × 2 ml	5 × 2 ml
Reagent Strip R[a]	16 (pre-filled, sealed)	96 (pre-filled, sealed)	--
Reagent Plate R[b]	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 (Strips)	2	12	5 × 12
Elution Strips	2	5 × 12	5 × 12
Manual	1	1	1

6.2 Components not included in the kit

- 1.5 ml tubes
- 2.0 ml tubes, optional
- 1 M DTT solution; optional

7 Initial steps before starting

- Add the indicated amount of ddH_2O to each vial of lyophilized **Proteinase K**, mix thoroughly and store as describes above.

845-IPS-2416016 845-IPP-2416016	Add 0.3 ml ddH2O to the vial of lyophilized Proteinase K.
845-IPS-2416096 845-IPP-2416096	Add 1.5 ml ddH2O to the vial of lyophilized Proteinase K.
845-IPP-2416480	Add 1.5 ml ddH2O to the vial of lyophilized Proteinase K.

- Ensure that the **Carrier Mix** and **Lysis Solution CBV / Carrier Mix** have been prepared according to the instruction (→ "Usage of Carrier Mix", p. 12).
- Heat thermal mixer or water bath to 50 °C.
- Centrifugation steps should be carried out at room temperature.
- 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:

- Swabs from different surfaces (e.g. cups, bottles, fingerprints)
- Blood samples
- Sperm samples
- Hair, hair roots or barb hairs
- Envelopes
- Finger nails
- 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/ External Lysis 200µl – Fast – 05	200 µl	43 / 41 min	20–500 µl

3. Typical yield:

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

9 Usage of Carrier Mix

9.1 General information on handling

Besides carrier RNA, the **Carrier Mix** contains an 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 / Carrier Mix.**

9.2 Preparation of Lysis Solution CBV / Carrier Mix mixture

1. Make sure that the Carrier Mix was prepared properly according to the instructions.
2. Prepare mixture of **Lysis Solution CBV / Carrier Mix** according to the table below

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

3. Store the mixture at 4–8 °C for a maximum of 7 days.

10 Protocols for isolation of DNA

10.1 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 during the complete lysis time in the 1.5 ml tube. 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 (→ p. 12) 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 in the Lysis Solution during the lysis time!

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!

2. After lysis time remove the swab from the 1.5 ml tube and squeeze the swab on the wall of the tube to remove all Lysis Solution CBV from the swab.
3. Proceed with automated extraction (→ “Preparing Reagent Plate / Strip for automated extraction”, p. 16).

10.2 Protocol 2: Extraction from sperm samples, hair roots, barb hairs, finger nails

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 (→ p. 12),

   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 in the Lysis Solution during the lysis time!

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the 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.

3. Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction", p. 16).

10.3 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 (→ p. 12),

   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

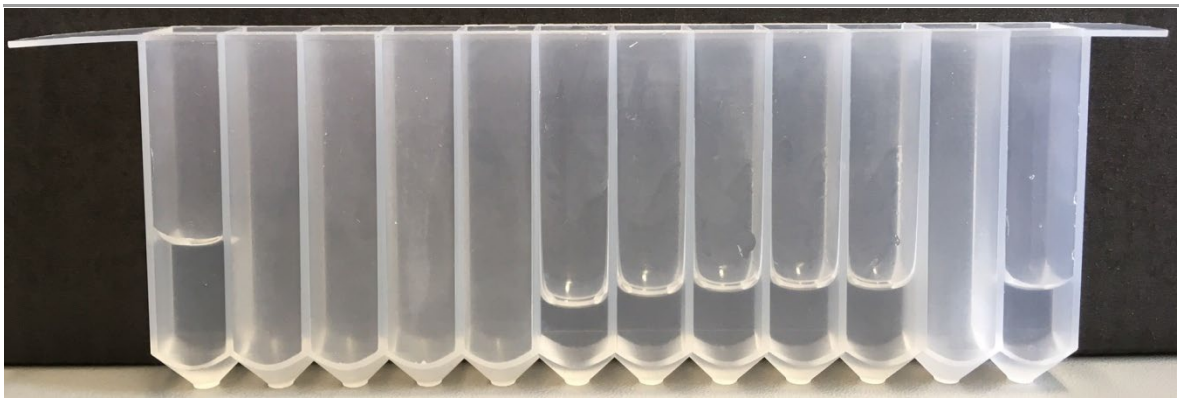
Assure that the sample is in the Lysis Solution during the lysis time!

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the 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.
3. Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction", p. 16).

11 Preparing Reagent Plate / Strip for automated extraction

11.1 General filling scheme of reagent reservoir



Cavity 1:	RNase-free Water	Cavity 7:	Washing Solution
Cavity 2:	Empty	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

11.2 Unpacking of Reagent Plate and piercing of sealing foil

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

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

Carefully open the overpack of Reagent Plates by using scissors.

11.3 Piercing of sealing foil of Reagent Plate or Reagent Strip

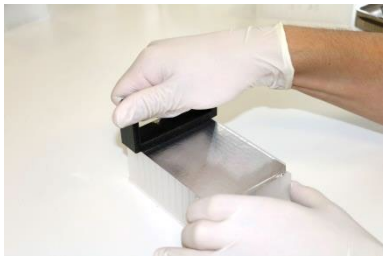
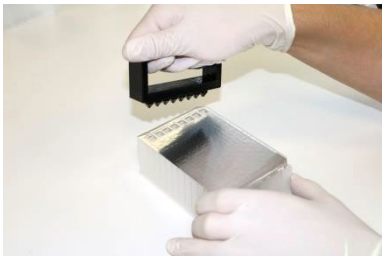
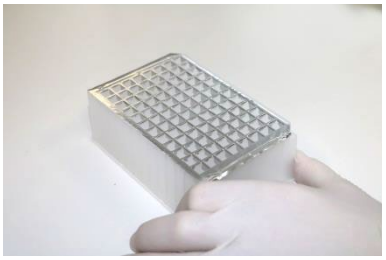
NOTE
Before using Reagent Plates or Strips the sealing foil has to be pierced manually. Always wear gloves while piercing of the foil!

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

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

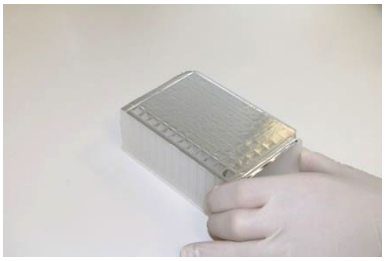
Open all cavities (one row per sample).

Using 8 samples in parallel

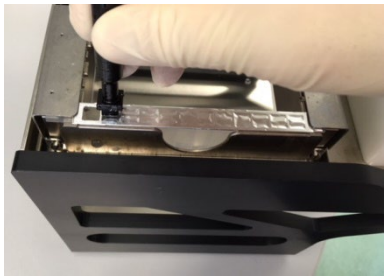
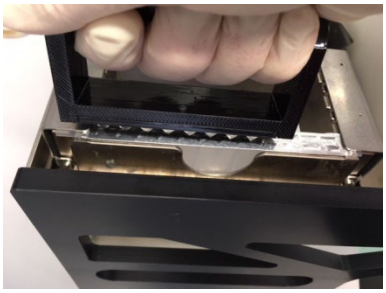
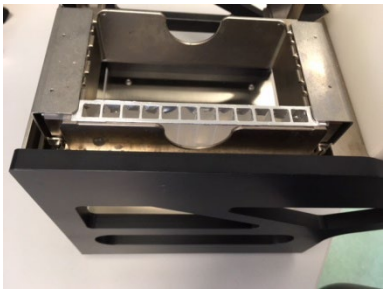




Using single samples





Using Reagent Strips

IMPORTANT

Use single or eightfold piercing tool for opening of all cavities of one row per sample!

11.4 Loading the sample to InnuPure C16 / C16 touch

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

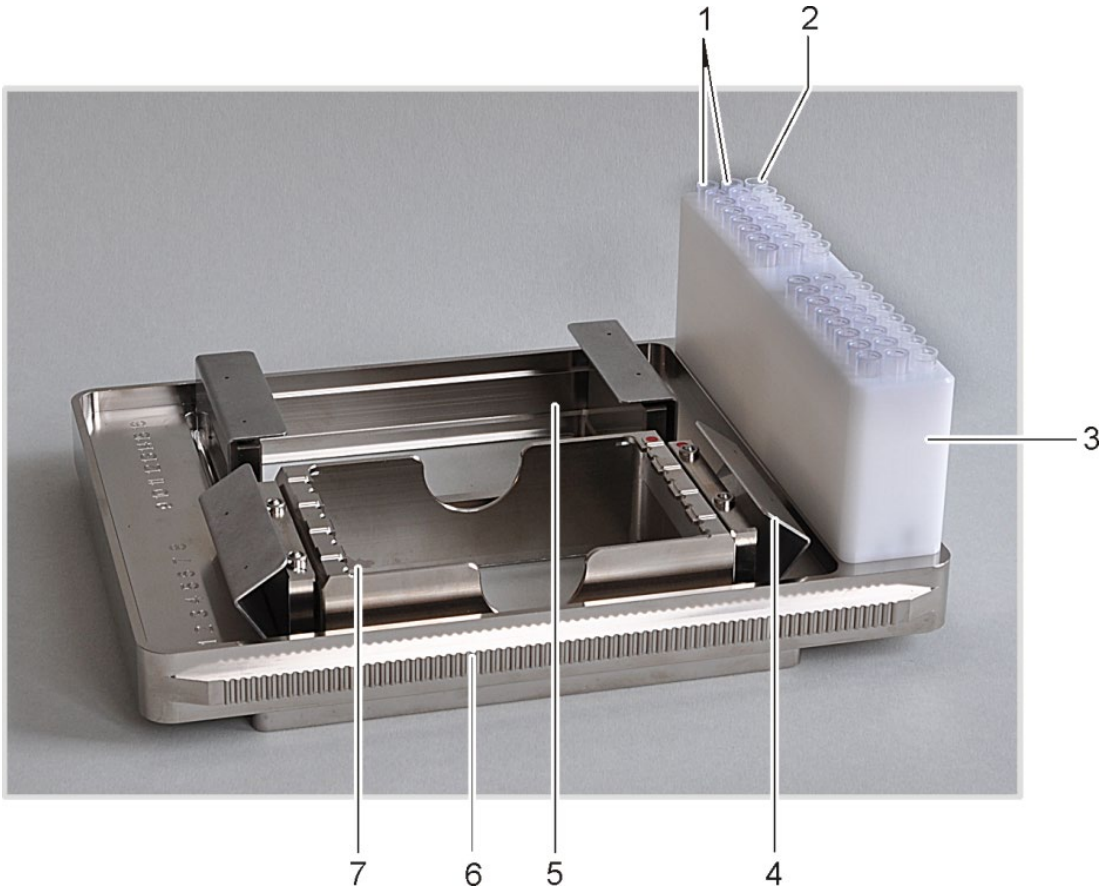
Ensure the foils of Reagent Plate or Reagent Strips have been pierced (→„Preparing Reagent / Strip for automated extraction“ p. 16).

1. Transfer **50 µl** of **MAG Suspension** directly into the liquid of the **first cavity** of Reagent Plate or Reagent Strip.
2. Transfer **400 µl** of the **lysed sample** into the **third cavity** of Reagent Strip or 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. 20.

12 Automated extraction using InnuPure C16 / C16 touch

12.1 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

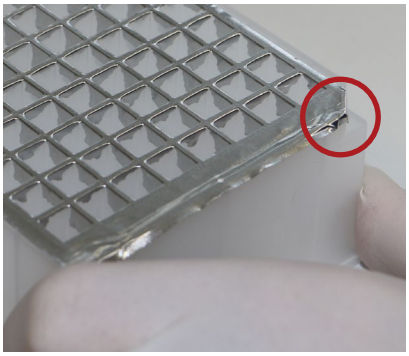
12.2 Preparing sample tray of InnuPure C16 / C16 touch

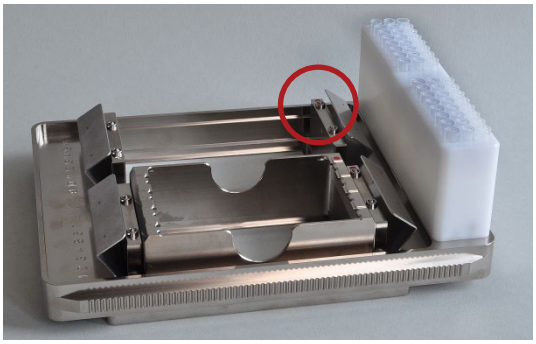
NOTE
The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more strips as number of samples!

1. Place 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 with Reagent Strips into the holder of the sample tray. Using Reagent Plates, the notched corner of the Reagent Plate has to align with the colored dot at the holder. Using adapters and Reagent Strips, the colored dot of the adapter has to align with the colored dot at the holder and Reagent Strips have to be inserted in a way that the “AJ” labels are arranged at the side of the adapter which is more distant from the tip block.

Reagent Plate

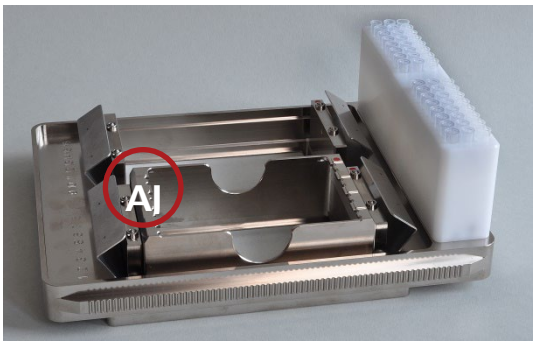
The notched corners of the Reagent Plate must point to the colored dot on the holder.





Reagent Strips

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

IMPORTANT NOTE
It is possible to select between two different elution vessels! For small elution volumes up to 200 µl use Elution Strips (0.2 ml). For high elution volumes up to 500 µl use Elution Tubes (0.65 ml) with corresponding Elution Caps (Strips).

12.3 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 Strips or Reagent Plates forward into the sample tray 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. 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 volume values from20 µl to 500 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 logfile. Find more detailed information how to start an extraction protocol using InnuPure C16 (→ user manual p. 37 "6.3.5 Using the sample setup tool")

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!

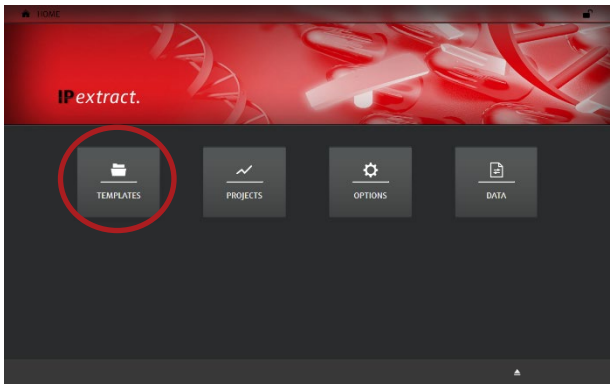
7. Remove the sample tray from the adapter of the InnuPure C16 and place it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

12.4 Starting the InnuPureC16 touch

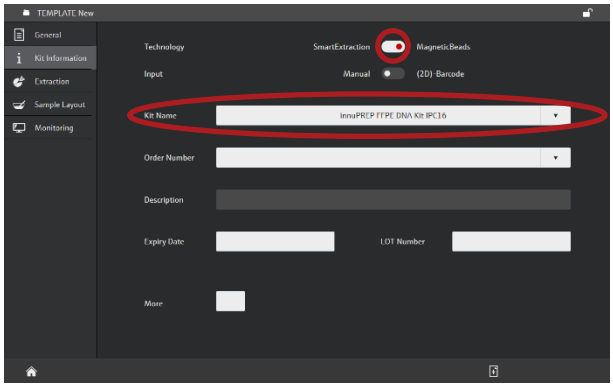
NOTE
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

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 “Kit Name”!



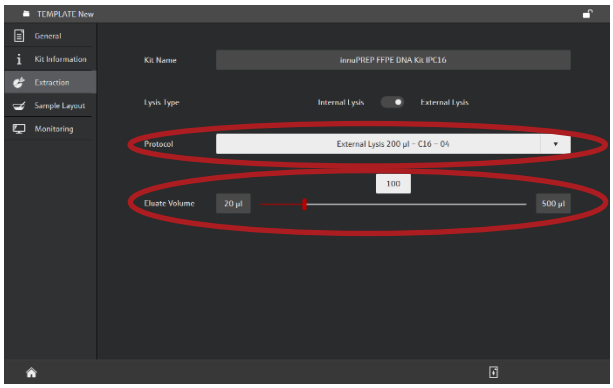
NOTE
“Kit Information” tab

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

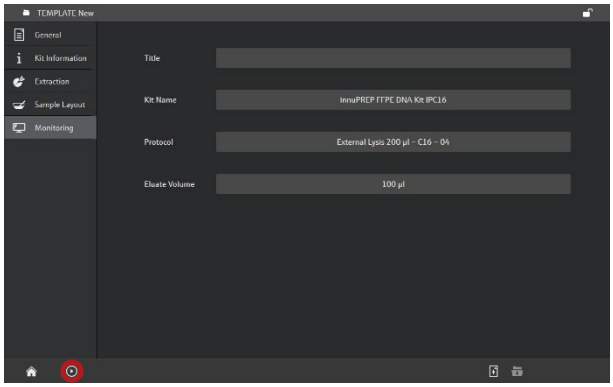
8. Adjust your desired “Eluate Volume“ using the slider or the text field.



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

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

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