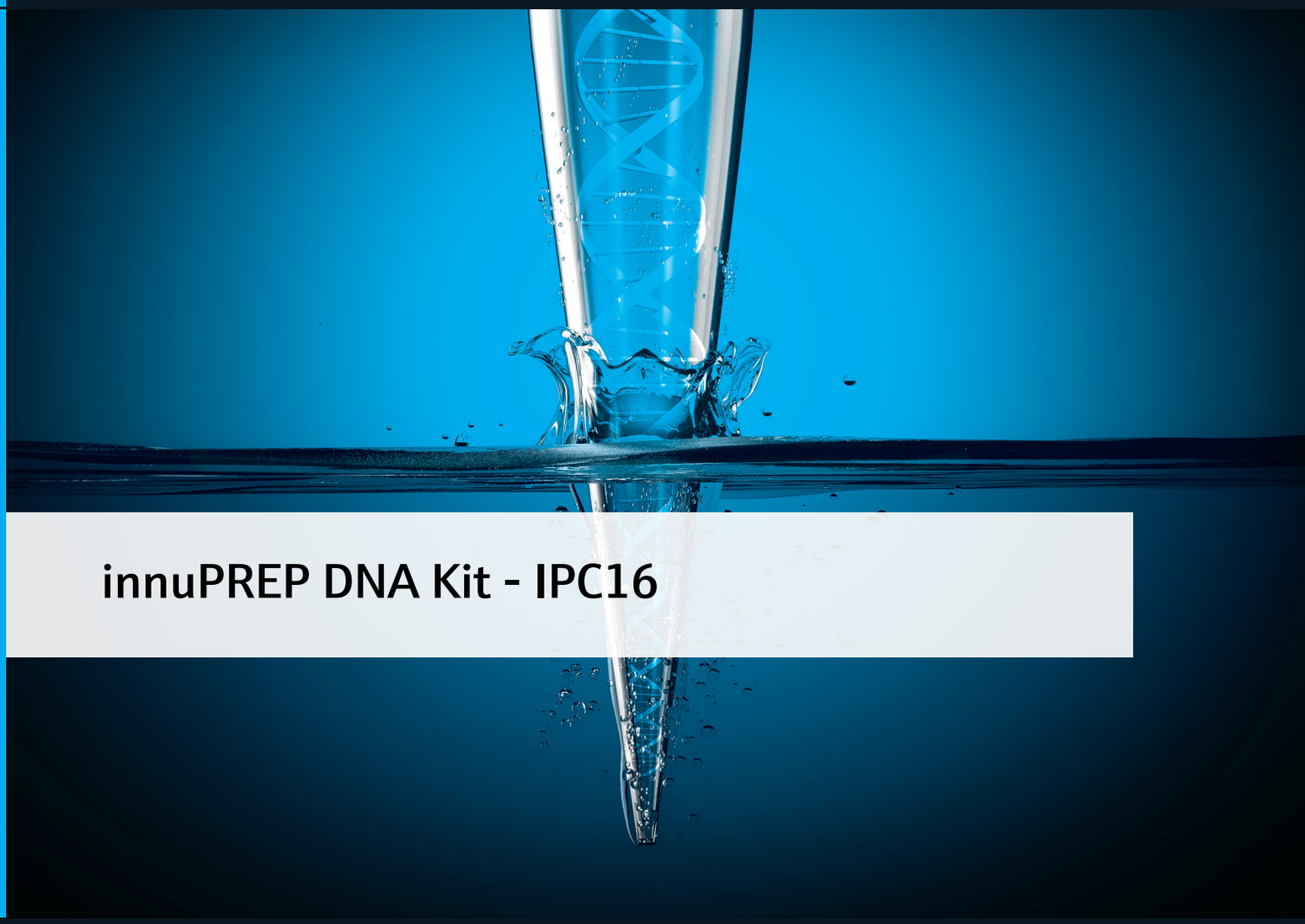
# Instructions for Use
Life Science Kits & Assays



## innuPREP DNA Kit - IPC16

Innuscreen GmbH

Order No.:

| | |
|---|---|
| 845-IPS-2016016 | 16 reactions |
| 845-IPP-2016016 | 16 reactions |
| 845-IPS-2016096 | 96 reactions |
| 845-IPP-2016096 | 96 reactions |
| 845-IPP-2016480 | 480 reactions |

Publication No.: HB_IP-2016_e_220107

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



**Manufacturer and Distributor:**
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!

Phone +49 30 9489 3380
Fax +49 30 9489 3381
info.innu@ist-ag.com

# Contents

# 1 Introduction

## 1.1 Intended use

The **innuPREP DNA Kit - IPC16** has been designed for automated isolation of DNA from eukaryotic cells, tissue samples, rodent tails and buccal swabs using the InnuPure C16 / C16 *touch*. The extraction procedure is based on a new-patented chemistry.

The procedure starts with an external lysis step. After the external lysis step the sample is transferred into the Reagent Strips or Reagent Plate of the kit, which is already prefilled with all extraction reagents needed for the extraction process. 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 particle with RNase-free water and is now ready to use for downstream applications. The extraction chemistry in combination with the InnuPure C16 / C16 *touch* protocol are optimized to get maximum of yield and quality.

**CONSULT INSTRUCTION FOR USE**

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

## 1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

| Symbol | Information |
|---|---|
| REF | **REF**<br>Catalogue number. |
| Σ N | **Content**<br>Contains sufficient reagents for <N> tests. |
| 15 °C 30 °C | **Storage conditions**<br>Store at room temperature, unless otherwise specified. |
| | **Consult instructions for use**<br>This information must be observed to avoid improper use of the kit and the kit components. |
| | **Expiry date** |
| LOT | **Lot number**<br>The number of the kit charge. |
| | **Manufactured by**<br>Contact information of manufacturer. |
| | **For single use only**<br>Do not use components for a second time. |
| | **Note / Attention**<br>Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results. |

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p. 3).
- Working steps are numbered.

# 2 Safety precautions

**NOTE**
Read through this chapter carefully before 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.

Store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C!

All other components of the **innuPREP 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.

# 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 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. 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.

# 6 Kit components

## 6.1 Included kit components

| | Σ 16 | Σ 96 | Σ 480 |
|---|---|---|---|
| REF | 845-IPS-2016016[a]<br>845-IPP-2016016[b] | 845-IPS-2016096[a]<br>845-IPP-2016096[b] | --<br>845-IPP-2016480[b] |
| **Lysis Solution CBV** | 10 ml | 50 ml | 250 ml |
| **Proteinase K** | For 2 × 0.3 ml working solution | For 2 × 1.5 ml working solution | For 7 × 1.5 ml working solution |
| **Reagent Strip A**[a] | 16<br>(pre-filled, sealed) | 96<br>(pre-filled, sealed) | -- |
| **Reagent Plate A**[b] | 2<br>(pre-filled, sealed) | 12<br>(pre-filled, sealed) | 60<br>(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 |

## 6.2 Components not included in the kit

- dd$H_2O$ for dissolving **Proteinase K**
- 1.5 ml tubes
- 2.0 ml tubes, optional
- RNase A (10 mg/ml); optional
- Buccal swabs

# 7 Initial steps before starting

- Add the indicated amount of dd$H_2O$ to **Proteinase K**, mix thoroughly and store as described above. and store as described above.

| | |
|---|---|
| 845-IPS-2016016 | Add 0.3 ml dd$H_2O$ to lyophilized Proteinase K. |
| 845-IPP-2016016 | Add 0.3 ml dd$H_2O$ to lyophilized Proteinase K. |
| 845-IPS-2016096 | Add 1.5 ml dd$H_2O$ to lyophilized Proteinase K. |
| 845-IPP-2016096 | Add 1.5 ml dd$H_2O$ to lyophilized Proteinase K. |
| 845-IPP-2016480 | Add 1.5 ml dd$H_2O$ to lyophilized Proteinase K. |

- 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.
- Avoid freezing and thawing of starting material.

# 8 Product specifications

1. Starting material:
   - Tissue samples (max. 20 mg)
   - Tissue samples with a high DNA content (e.g. spleen samples, pancreatic samples, lymph nodes, max. 5 mg)
   - Mouse tails (up to 1.0 cm) and rat tails (up to 0.5 cm)
   - Eucaryotic cells (max. 5 x $10^6$)
   - Buccal swab samples

2. Time for isolation:

   External lysis steps

| | |
|---|---|
| Tissue and tail samples | approx. 45–75 minutes |
| Eucaryotic cells | approx. 10–20 minutes |
| Buccal swab samples | approx. 15 minutes |

| Extraction protocol | Protocol on InnuPure C16 / C16 touch | Time InnuPure C16 / 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:
   - Depending on type and amount of the starting material
   - Tissue samples and rodent tails: up to 50 µg
   - Eucaryotic cells: up to 25 µg
   - Buccal swabs: sufficient DNA for PCR applications

# 9 Protocols for DNA isolation

## 9.1 Protocol 1: Isolation from tissue samples or rodent tails

**NOTE**
Max. amount of tissue samples is 20 mg. Tissue samples with a high DNA content (e.g. spleen samples, pancreatic samples, lymph nodes) use max. 5 mg.
Max. rodent tails: mouse tail (1.0 cm); rat tail (0.5 cm).

1. Cut tissue sample or the rodent tail into small pieces and place the sample into a 1.5 ml reaction tube.
2. Add to sample:

   **200 µl $ddH_2O$,**

   **200 µl Lysis Solution CBV and**

   **20 µl Proteinase K**

Incubate at 55 °C in a shaking platform until the sample is lysed. Sample lysis time depends on amount and kind of sample. Lysis should be completed within 0.5–3 hours.

**NOTE**
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!
To remove RNA from the sample (optional) add 1–2 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 5 minutes at room temperature.

3. Centrifuge the 1.5 ml tube at 10,000 x g (~12,000 rpm) for 2 minute to spin down unlysed material.
4. Proceed with automated extraction (→ " Preparing Reagent Plate / Strip for automated extraction ", p. 14).

## 9.2 Protocol 2: Isolation from eucaryotic cell (max. 5 x $10^6$ cells)

1. Pellet eucaryotic cells by centrifugation for 10 minutes at 5,000 x g (~7,500 rpm). Discard supernatant.
2. Add **200 µl dd$H_2O$** to the cell pellet and resuspend the cell pellet completely by pipetting up and down.
3. Add **200 µl Lysis Solution CBV** and **20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds. Incubate at 50 °C until the sample is completely lysed (approx. 20 minutes).

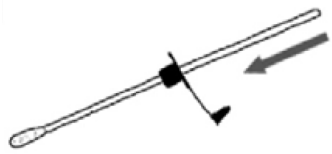
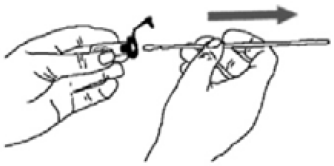
**NOTE**
We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!
To remove RNA from the sample (optional) add 1–2 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 5 minutes at room temperature.

4. Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction ", p. 14).

## 9.3 Protocol 3: Isolation from buccal swabs

### 9.3.1 Instructions for use of buccal swabs

**NOTE**
Intended for the retrieval of buccal cells. Single use only.
Store at room temperature. Use only if swab wrapper remains intact.
Follow the correct sampling procedure to avoid risk of the swab head becoming detached in the mouth.
If the swab head does become detached from the swab stick whilst in the mouth, remove immediately.

| | |
|---|---|
|  | Pull open the package from one end. |
|  | Remove the swab from the tube *by turning*, taking care not to touch the white swab head with your fingers. |
|  | Insert the swab into your mouth and rub firmly against the inside of your cheek or underneath lower and upper lip. For standard DNA collection rub for 1 minute and in all cases rub for a minimum of 20 seconds. We rcommend rub for 1 minute.<br>Important – use reasonable, firm and solid pressure! |
|  | Slide the plastic cap over the swab handle with the flat side of the cap facing upwards and the swab facing downwards. |
|  | Insert the swab into the clear plastic tube and push the cap into place.<br>Next, hold the cap while pulling the swab handle outwards to release the swab material into the tube. |
|  | Close the cap by pushing the stopper fully into the cap ensuring the stopper is flush with the cap.<br>The tube is now completely sealed. |

### 9.3.2 Lysis of buccal swab

1. Open the cap of the buccal swab tube and add:

   **300 µl $ddH_2O$,**

   **300 µl Lysis Solution CBV and**

   **20 µl Proteinase K**

2. 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!

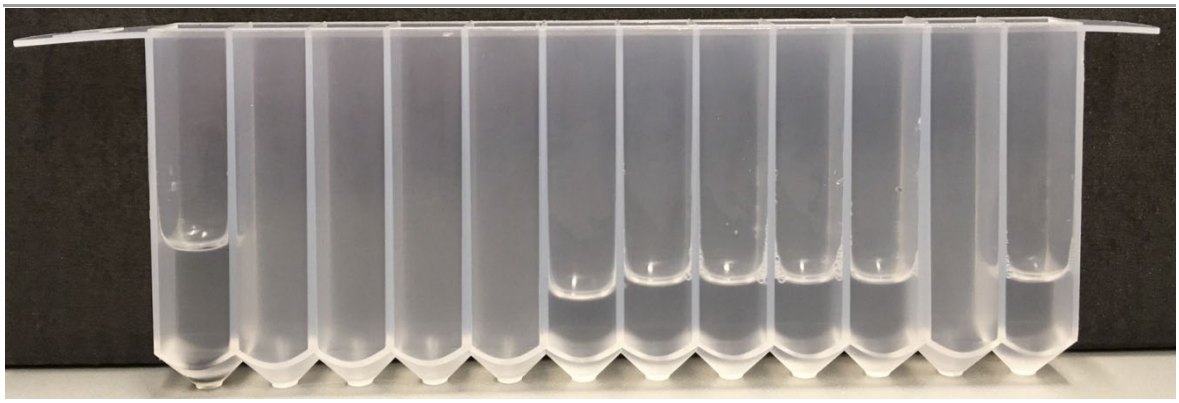
We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!
To remove RNA from the sample (optional) add 1–2 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 5 minutes at room temperature.

3. After lysis mix vigorously by pulsed vortexing for 20 seconds.

Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction ", p. 14).

# 10 Preparing Reagent Plate / Strip for automated extraction

## 10.1 General filling scheme of reagent reservoir



| | | | |
|---|---|---|---|
| Cavity 1: | Magnetic particles | 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 |

## 10.2 Unpacking of Reagent Plate or Reagent Strip

**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 or Strips by using scissors.

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

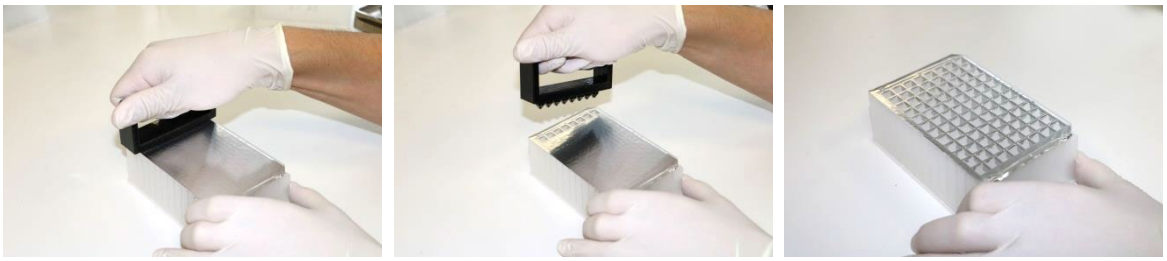
**NOTE**
Before using Reagent Plates or Strips the sealing foil has to be pierced manually. Always wear gloves while piercing of the foil!

Reagent Plates or Strips are prefilled with extraction reagents and are sealed with a foil. This foil has to be pierced manually before use, by 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.

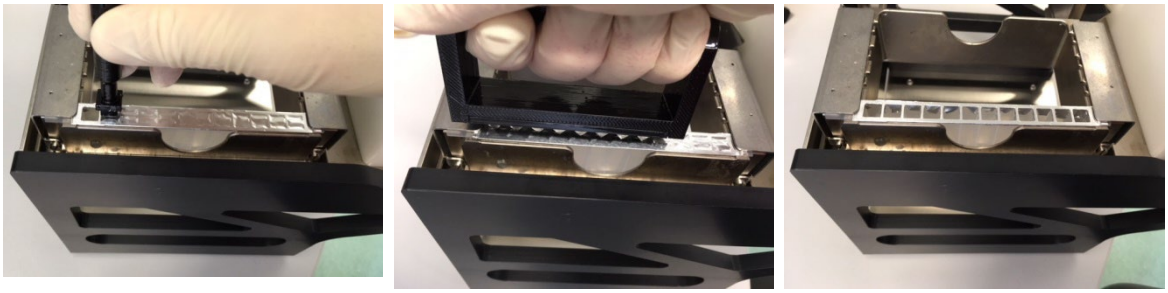
Open all cavities (one row per sample).

**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!

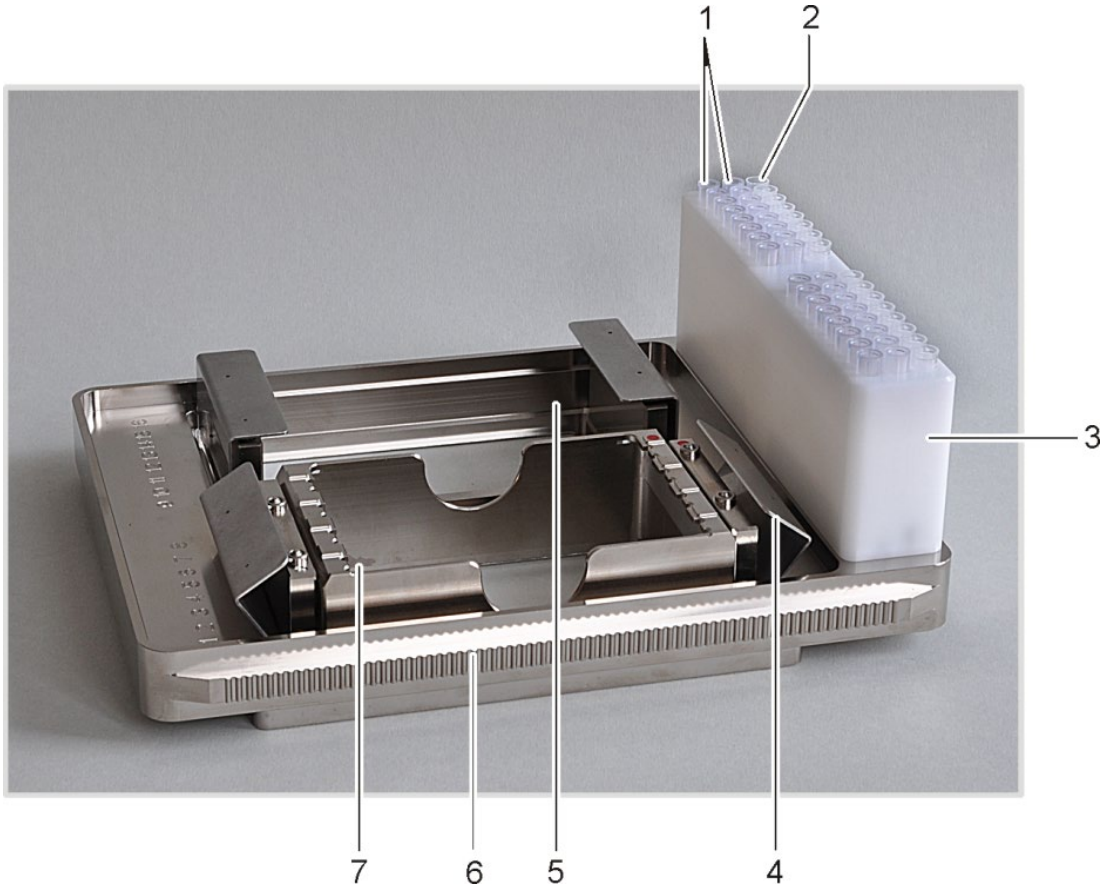
## 10.4 Loading the sample to InnuPure C16 / C16 touch

1. Ensure the foils of Reagent Plate or Reagent strips have been pierced (→„Preparing Reagent Plate / Strip for automated extraction“ p. 14).
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 12 p. 25.

# 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 |

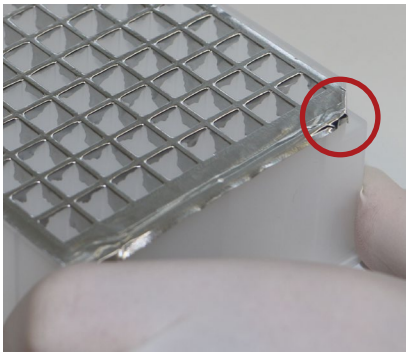
## 11.2 Preparing sample tray of InnuPure C16 / C16 touch

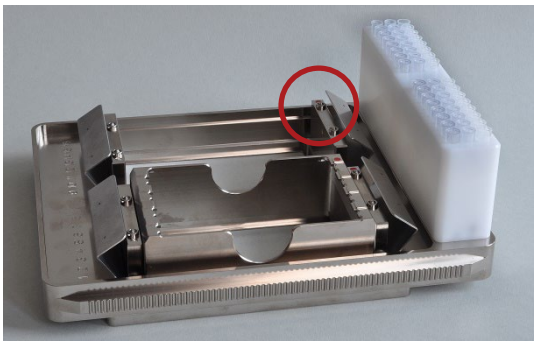
**NOTE**
The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more strips as number of samples!

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**

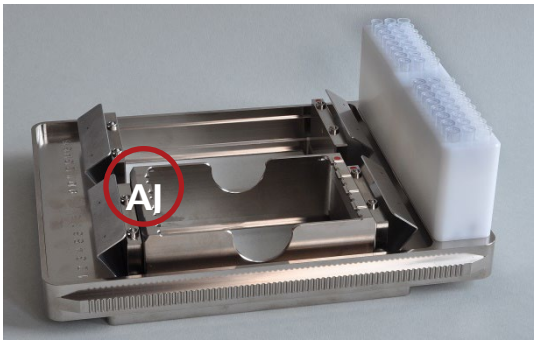
The notched corners of the Reagent Plate must point to the colored dot on the holder.





**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 (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 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 InnuPure C16 |
|---|---|
| **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** (Protocol 1 and 2) or **100 µl** for Protocol 3 (Buccal swabs) 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 on page 37 of the user manual "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!

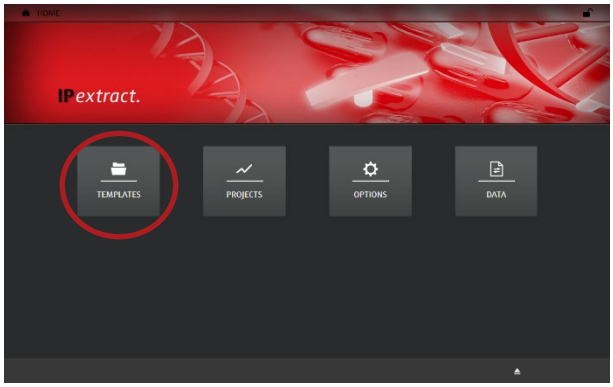
7. Remove the sample tray from the adapter of the InnuPure C16 and place it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

## 11.4 Starting the InnuPure C16 touch

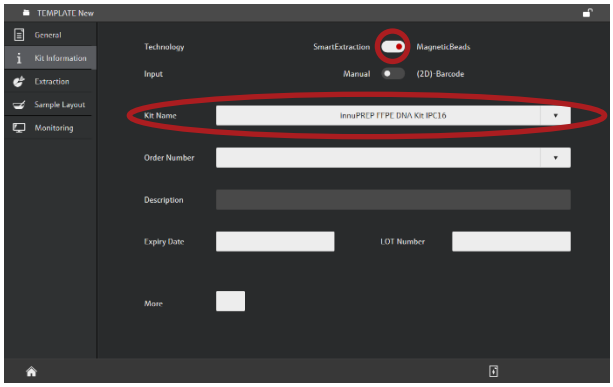
**NOTE**
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

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 “Kit Name”!



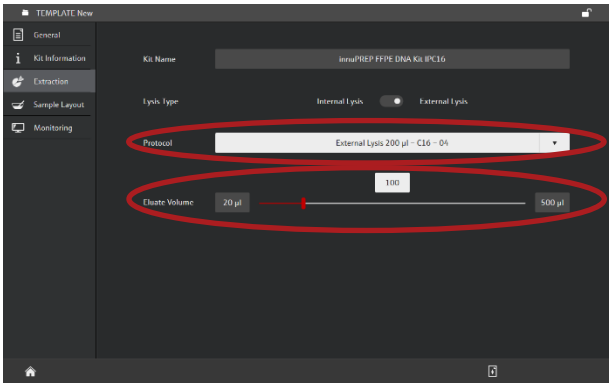

**NOTE**
“Kit Information” tab

6. Enter optional information in the tab “Kit Information”

7. Choose the tab “Extraction” and choose the desired “Protocol”

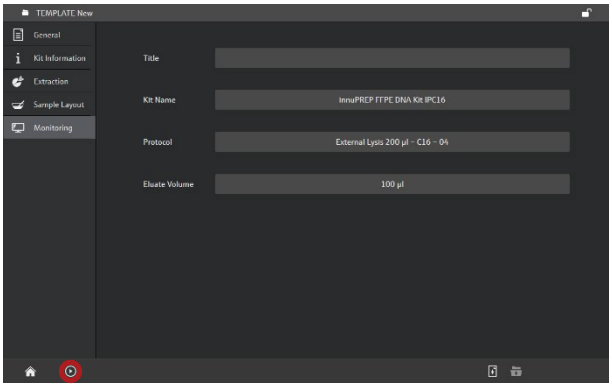
| Extraction procedure | Protocol on InnuPure C16 touch |
|---|---|
| **Standard**<br>(maximum yield, approx. 52 minutes) | External Lysis 200 µl - 05 |
| **Fast**<br>(time-optimized, approx. 41 minutes) | External Lysis 200 µl - Fast - 05 |

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




**NOTE**
“Extraction” tab
**The recommended elution volume is 200 µl for Protocol 1 and 2.**
**The recommended elution volume is 100 µl for Protocol 3 (Buccal swabs).**

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!

# 12 Troubleshooting

| Problem / probable cause | Comments and suggestions |
|---|---|
| **Low amount of extracted genomic DNA** | |
| No extracted DNA | Ensure that the **Proteinase K** has 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. |
| Elution volume too high | 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! |
| Eluate exert high viscosity | Elution volume to low. Increase the elution volume. The suggested elution volume is 200 µl up can be up to 500 µl. |

IST Innuscreen GmbH
Robert-Rössle-Str.10
13125 Berlin · Germany

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

IST Innuscreen GmbH