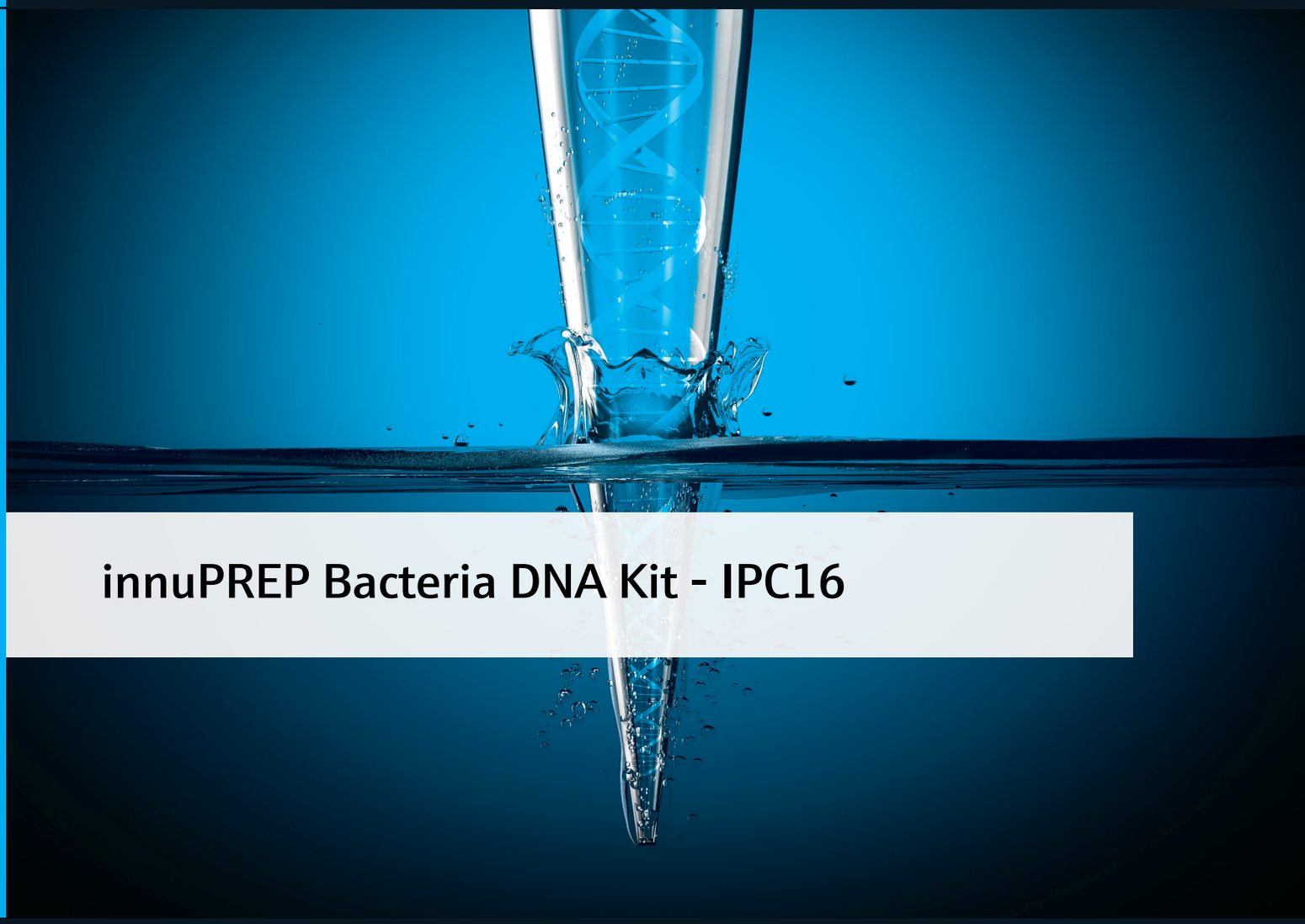
Instructions for Use
Life Science Kits & Assays



innuPREP Bacteria DNA Kit - IPC16

Innuscreen GmbH

Order No.:

845-IPS-5516016	16 reactions
845-IPP-5516016	16 reactions
845-IPS-5516096	96 reactions
845-IPP-5516096	96 reactions
845-IPP-5516480	480 reactions

Publication No.: HB_IP-5516_e_220107

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 Bacteria DNA Kit - IPC16** has been designed for automated isolation of DNA from Gram-positive and Gram-negative bacteria using the InnuPure C16 / C16 *touch*. The extraction procedure is based on a new-patented chemistry.

The procedure combines an external lysis step of the bacteria cells using lysozyme with a subsequent proteolytic digestion step. After the 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. The extraction chemistry in combination with the InnuPure C16 / C16 *touch* protocol are optimized to get maximum of yield and quality.

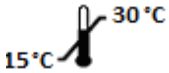
CONSULT INSTRUCTION FOR USE

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** Catalogue number.
Σ	**Content** Contains sufficient reagents for <N> tests.

Symbol	Information
	**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. 2).
- 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 Bacteria 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

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 Bacteria 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 (→ “Product specifications” p. 8). 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 di-

agnostic 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-5516016[a] 845-IPP-5516016[b]	845-IPS-5516096[a] 845-IPP-5516096[b]	-- 845-IPP-5516480[b]
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 x 1.5 ml working solution
Reagent Strip A[a]	16 (pre-filled, sealed)	96 (pre-filled, sealed)	---
Reagent Plate A[b]	2 (pre-filled, sealed)	12 (pre-filled, sealed)	60 (pre-filled, sealed)
Filter Tips	2 × 16	2 × 96	10 x 96
Elution Tubes (0.65 ml)	16	2 × 48	10 x 48
Elution Caps (Stripes)	2	12	5 x 12
Elution Strips	2	12	5x 12
Manual	1	1	1

6.2 Components not included in the kit

- ddH_2O for dissolving **Proteinase K**
- TE Buffer (1 mM EDTA; 10 mM Tris HCl, pH 7.5)
- RNase A (10 mg/ml); optional
- 1.5 ml tubes
- 2.0 ml tubes, optional
- Lysozym (10 mg/ml), Mutanolysin (stock solution: 0.4 U/µl), Lysostaphin (stock solution: 0.4 U/µl); optional OR
- Alternatively: innuPREP Bacteria Lysis Booster (845-KA-1000050)

7 Initial steps before starting

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

845-IPS-5516016 845-IPP-5516016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-IPS-5516096 845-IPP-5516096 845-IPP-5516480	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Heat thermal mixer or water bath to 37 °C and 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:
   - Bacterial cell pellets (maximum 1 x 10^9 cells)
2. Time for isolation:
   - External lysis step approx. 35–45 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

9 Protocol for isolation of DNA from bacterial cells

9.1 Pre-lysis of resuspended starting material

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g) and discard the supernatant.

2. Resuspend the bacteria cell pellet in **200 µl TE Buffer**. After resuspension start enzymatic pre-lysis as described below. Requirements for pre-lysis depend on the cell type.

9.1.1 Gram-negative bacteria

Although Gram-negative bacteria do not require a pre-lysis step, using Lysozyme (not included in the kit) can enhance the efficiency of lysis.

Using Lysozyme

Stock solution of Lysozyme: 10 mg/ml (400 U/µl)

Add **20 µl Lysozyme** to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.

Proceed with “Proteolytic lysis step” on p. 10.

9.1.2 Gram-positive bacteria

Gram-positive bacteria require a pre-lysis step using Mutanolyis and/or Lysozyme (not included in the kit).

Using Lysozyme

Stock solution of Lysozyme: 10 mg/ml (400 U/µl)

Add **20 µl Lysozyme** to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.

Using Mutanolysin

Stock solution of Mutanolysin: 0.4 U/µl

Add **5 µl Mutanolysin** to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.

Proceed with “Proteolytic lysis step” on p. 10.

NOTE

Lysozyme and Mutanolysin exert synergistic activity. Using both enzymes together will increase the yield of isolated nucleic acids.

Alternatively:

Use the **innuPREP Bacteria Lysis Booster**

The innuPREP Bacteria Lysis Booster Kit has been developed for a highly efficient pre-lysis of bacterial cell walls by generating spheroblasts. This new mixture of different enzymes boosts the lysis of all bacteria in particular hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium*.

Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.

Add **20 µl** of the prepared **enzyme mix** to the sample and vortex it shortly. Incubate the sample for 30 minutes at 37 °C.

Proceed with “Proteolytic lysis step” on p. 10.

9.1.3 Staphylococcus

For lysis of *Staphylococcus* the enzyme Lysostaphin is recommended (not included in the kit)

Stock solution of Lysostaphin: 0.4 U/µl

Add **10 µl Lysostaphin** to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.

Proceed with “Proteolytic lysis step” on p. 10.

Alternatively:

Use the **innuPREP Bacteria Lysis Booster**

The innuPREP Bacteria Lysis Booster Kit has been developed for a high efficient pre-lysis of bacterial cell walls by generating sphaeroblasts. This new mixture of different enzymes boosts the lysis of all bacteria in particular hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium*.

Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.

Add **20 µl** of the prepared enzyme mix to the sample and vortex it shortly. Incubate the sample for 30 minutes at 37°C.

Proceed with “Proteolytic lysis step” on p. 10.

9.2 Proteolytic lysis step

1. Add **200 µl Lysis Solution CBV** and **20 µl Proteinase K** to the sample and mix vigorously by pulsed vortexing for 5 seconds.

2. Incubate sample for 30 minutes at 50°C and 550 rpm in a shaking platform.

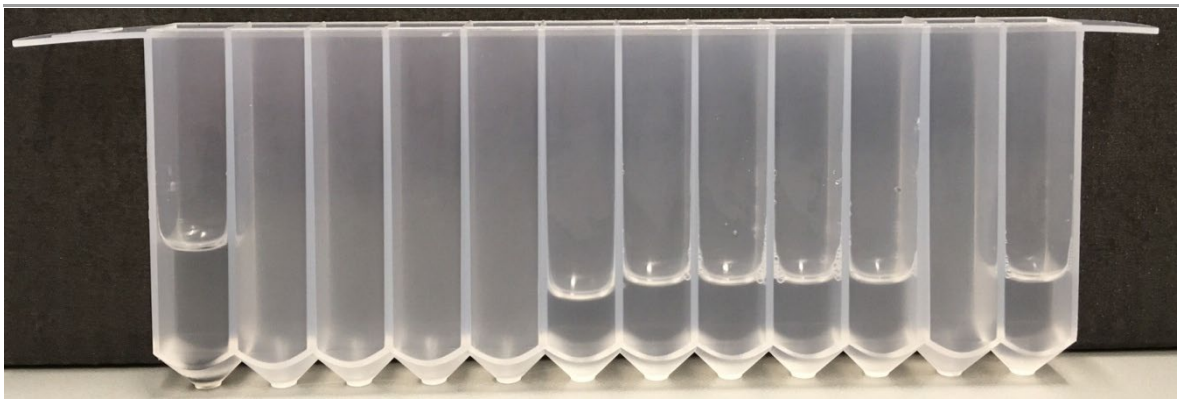
   Lysis time of 30 minutes is often sufficient to get enough DNA. If the sample is not clear after 30 minutes prolong the incubation time until the sample is clear.

NOTE
To remove RNA from the sample (optional) add 1 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 10 minutes at room temperature. Be sure, that the RNase A is free of DNase-activity.

3. Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction ", p. 11).

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

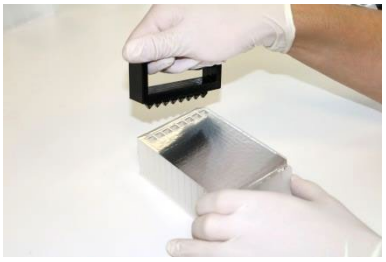
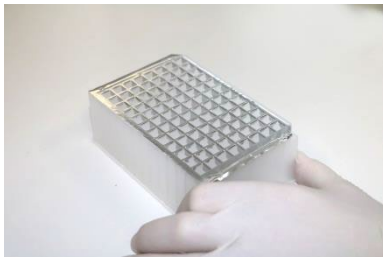
NOTE
Before using Reagent Plates or Strips the sealing foil has to be pierced manually. Always wear gloves while piercing of the foil!

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.

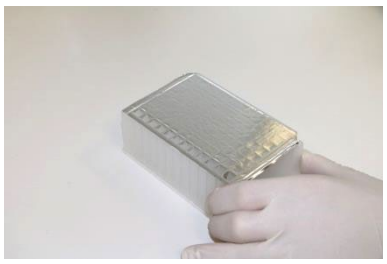
Open all cavities (one row per sample).

Using 8 samples in parallel

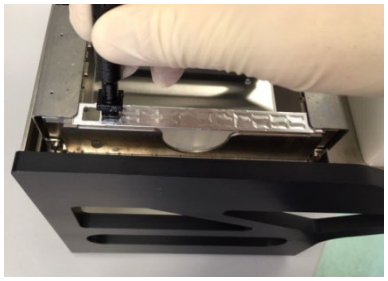
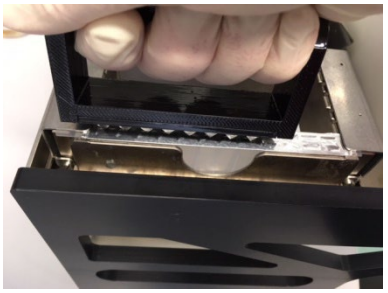
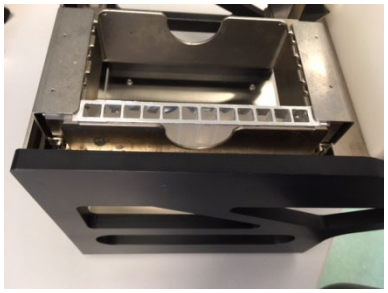




Using single samples





Using Reagent Strips

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

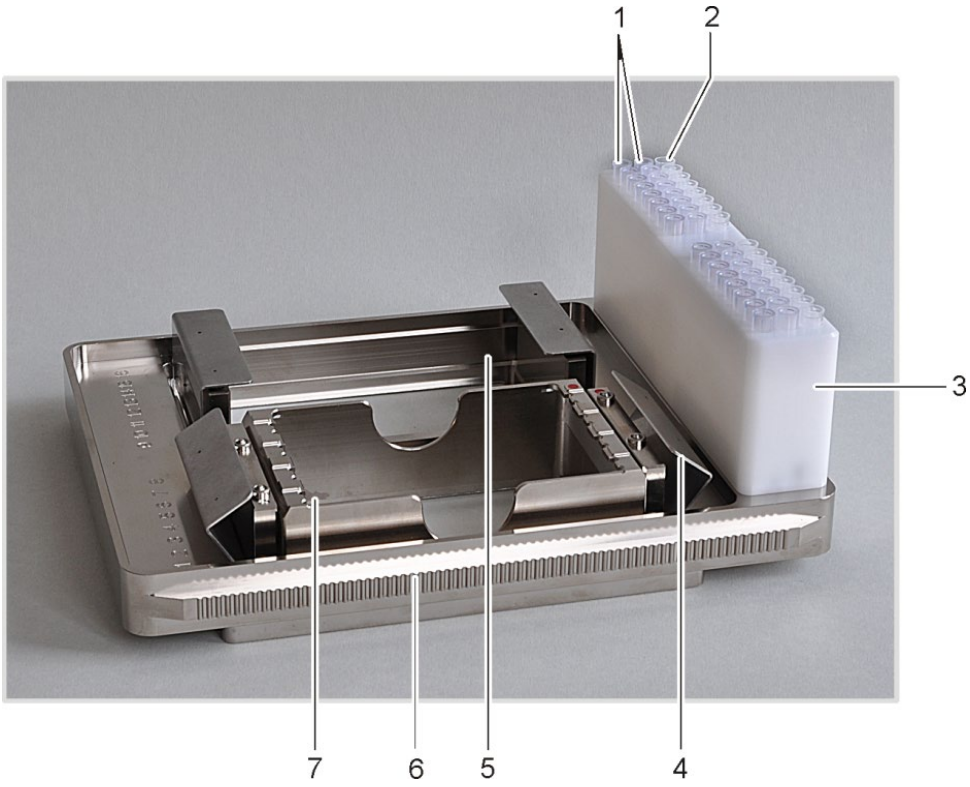
10.4 Loading the sample to InnuPure C16 / C16 touch

1. Ensure the foils of Reagent Plate or Reagent Strips have been pierced (→„Preparing Reagent Plate / Strip for automated extraction“ p. 11).
2. Transfer **400 µl** of the **lysed sample** into the **third cavity** of Reagent Plate or Reagent Strip. 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. 14.

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

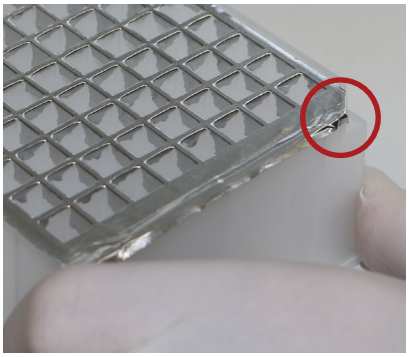
11.2 Preparing sample tray of InnuPure C16 / C16 touch

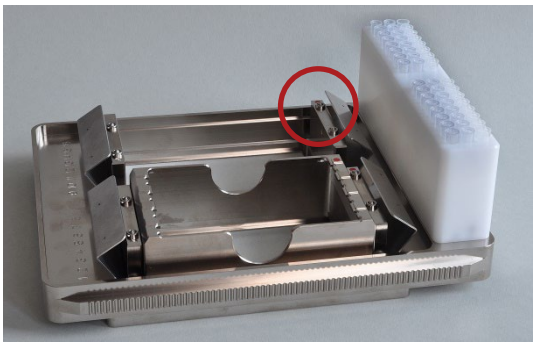
NOTE
The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more strips as number of samples!

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

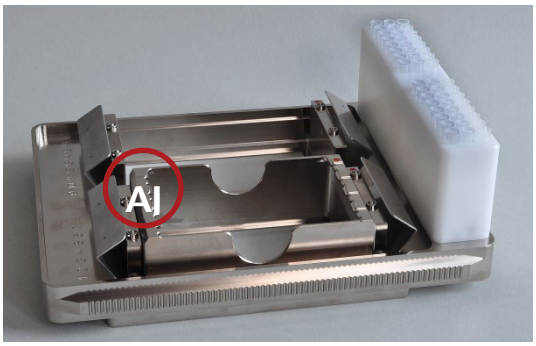
The colored line of the reagent plate must must point to the colored dot on the holder





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.

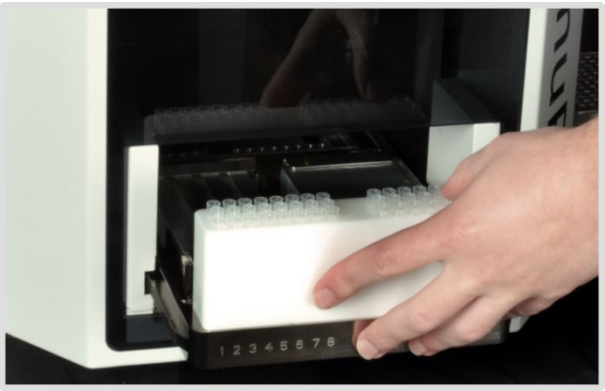
3. Fold down the holding-down clamp to prevent the Reagent Plates and Reagent Strips to be pulled out of the holder during the extraction process.
4. For each extracted sample place two filter tips in the smaller drill holes of the tip block.
5. Place the Elution Tubes into the wider drill hole at the 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 **100–150 µl** and press [OK].

NOTE
It is possible to adjust the volume values from 20 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 set-up 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!

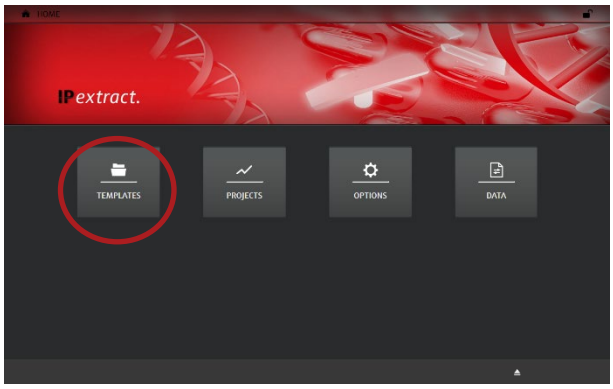
7. Remove the sample tray from the adapter of the InnuPure C16 and place it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

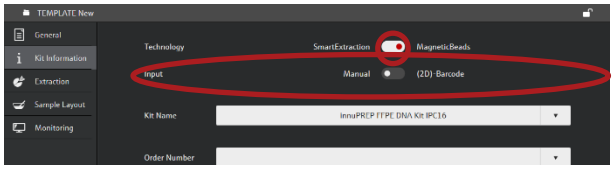
NOTE
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

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”!

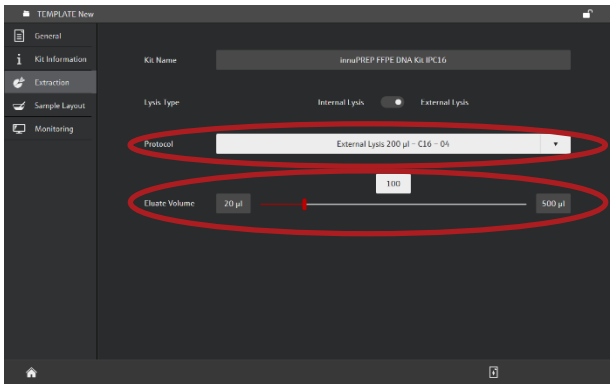


NOTE
“Kit Information” tab

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 (maximum yield, approx. 52 minutes)	External Lysis 200 µl - 05
Fast (time-optimized, approx. 41 minutes)	External Lysis 200 µl - Fast - 05

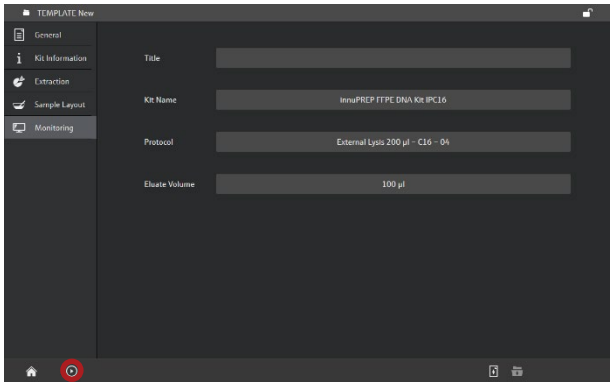
8. Adjust your desired “Eluate Volume“ using the slider or the text field.



NOTE
“Extraction” tab

The recommended elution volume is 100–150 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!

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.
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.

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