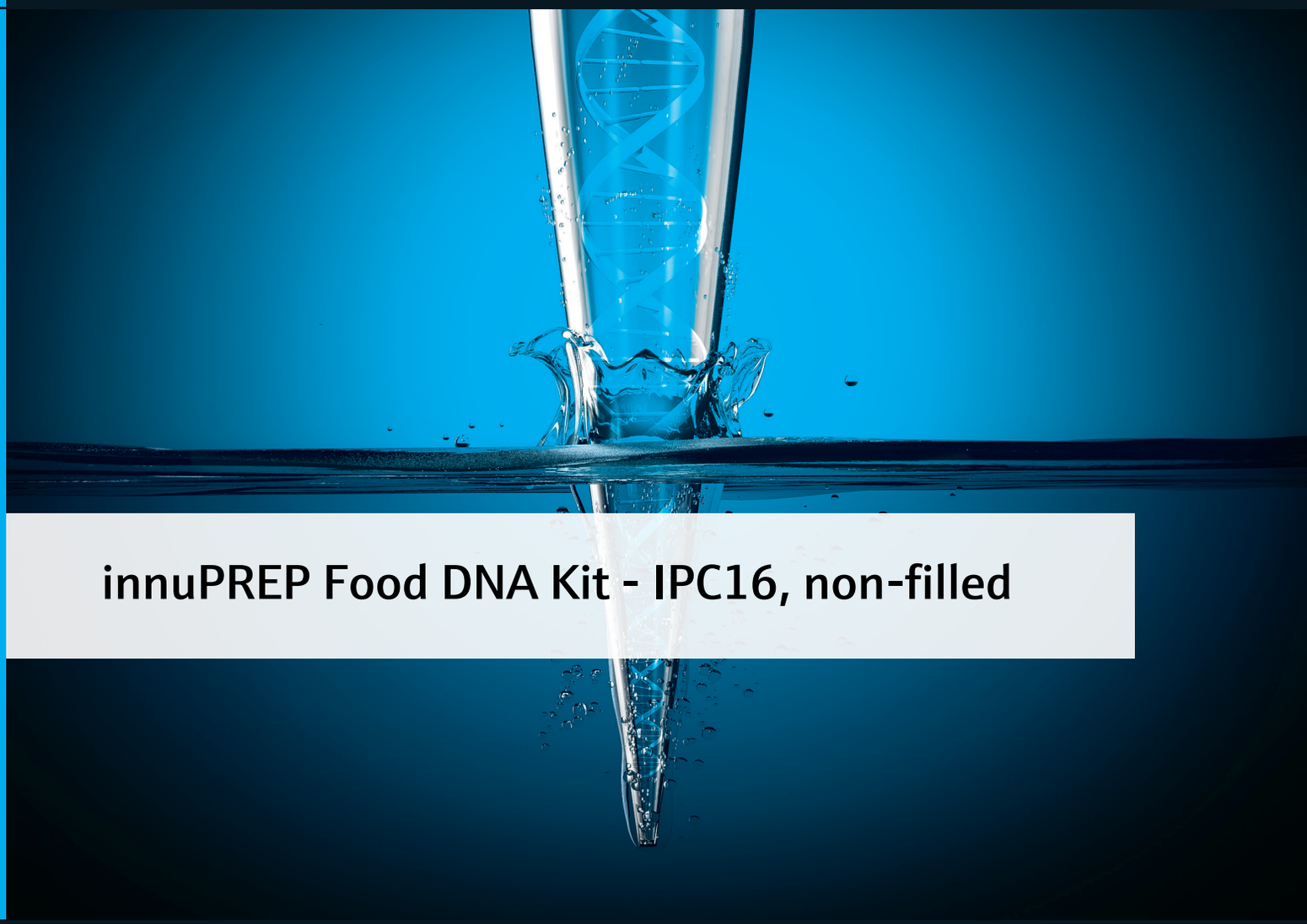
Instructions for Use
Life Science Kits & Assays



innuPREP Food DNA Kit - IPC16, non-filled

Innuscreen GmbH

Order No.:
845-PPP-5716016 16 reactions
845-PPP-5716096 96 reactions
845-PPP-5716480 480 reactions

Publication No.: HB_PPP-5716_e_230213

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1 Introduction

1.1 Intended use

The **innuPREP Food DNA Kit - IPC16, non-filled** has been designed for automated isolation of DNA from food samples using the InnuPure C16 / C16 *touch*. The extraction procedure is based on a new-patented chemistry.

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

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	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> tests.
15 °C 30 °C	**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	**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 is designed to 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. This kit is to be used with potentially infectious human samples. 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 lyophilized and dissolved **Proteinase K** and **MAG Suspension** at 4 °C to 8 °C.

All other components of the **innuPREP Food 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 Food 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 (→ “Intended use”, p. 2) (→ “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.

6 Kit components

6.1 Components included in the kit

	Σ 16	Σ 96	Σ 480
REF	845-PPP-5716016	845-PPP-5716096	845-PPP-5716480
Lysis Solution CBV	25 ml	2x 120 ml	3x 250 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution	for 7 x 1.5 ml working solution
Deep Well Plate (2.0 ml)	2 (empty)	12 (empty)	60 (empty)
MAG Suspension	1.5 ml	5.5 ml	3 x 9 ml
Binding Solution SBS	2 x 5 ml	60 ml	4 x 70 ml
Washing Solution E	12 ml	2 x 35 ml	3 x 100 ml
Washing Solution B2 (conc.)	16 ml	80 ml	8 x 48 ml
RNase free Water	30 ml	2 x 100 ml	4 x 200 ml
Filter Tips	2 x 16	2 x 96	10 x 96
Elution Tubes (0.65 ml)	16	2 x 48	10 x 48
Elution Caps (Stripes)	2	12	5 x 12
Elution Strips	2	12	5 x 12
Manual	1	1	1

6.2 Components not included in the kit

- 96 %–99.8 % Ethanol (molecular biology grade, non-denaturated)
- ddH_2O for dissolving **Proteinase K**
- RNase A (10 mg/ ml), optional

- 1.5 ml and 2.0 ml tubes

6.3 Related Products

- Deep Well Plate (96 square well, 2.0 ml 845-FX-8500025, 25 pcs)
- Deep Well Plate (96 square well, 2.0 ml 845-FX-8500115, 115 pcs)
- IPC16 Dummy Plate (sealed, 31-00258, 1 piece)

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-PPP-5716016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-PPP-5716096 845-PPP-5716480	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-5716016	Add 24 ml of **96-99.8 % ethanol** to 16 ml **Washing Solution B2 (conc.)**
845-PPP-5716096	Add 120 ml of **96-99.8 % ethanol** to 80 ml **Washing Solution B2 (conc.)**
845-PPP-5716480	Add 72 ml of **96-99.8 % ethanol** to 48 ml **Washing Solution B2 (conc.)**

- Avoid freezing and thawing of starting material.
- Pre-heat thermal mixer or water bath to 65 °C.
- Centrifugation steps should be carried out at room temperature.

8 Product specifications

1. Starting material:
   - Food samples (max. 200 mg)

2. Time for isolation:

   Manual steps:
   - Lysis: approx. 60 minutes
   - Processing after lysis: approx. 15 minutes

   Automated steps:

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

3. Typical yield:
   - Depending on amount and quality of starting material.

9 Protocol: Lysis of food samples

1. Weigh up to **200 mg** of food sample. Cut the sample into small pieces or homogenize the sample as much as possible before transferring it into a 2.0 ml tube.
2. Add the recommended amount of **Lysis Solution CBV** (see table) and **20 µl Proteinase K** to each sample and vortex vigorously for 10 seconds. Incubate at 65 °C for approx. 60 minutes.

Food class	Example	Amount of Lysis Solution CBV to be added to the sample
Meat products	ham, salami	0.8 ml
Tinned food	fish, meat or sausages	0.8 ml
Milk products	cheese, yoghurt, chocolate	0.8 ml
Cereals	flakes, nachos, waffles, cookies, noodles	1.5 ml
Flours	wheat flour, baking mixes	1.2 ml
Instant products	Instant soups, mashed potatoes	1.0 ml

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample every 10 minutes during the incubation. No shaking will reduce the lysis efficiency.

3. Centrifuge the tube at 11,000 x g for 10 minutes.
4. Transfer the supernatant into a new 1.5 ml reaction tube.

   If there is floating material above the sample, pierce this film carefully with a pipette and carefully remove the sample. Avoid aspiration of floating material and/or sediment.

NOTE

To remove RNA from the sample (if necessary) add 2 µl of RNase A solution (10 mg/ml) to the lysed sample, vortex shortly and incubate for 5 minutes at room temperature.

5. Check if the sample volume is at least **400 µl**. If it is lower, add **Lysis Solution CBV up to 400 µl**.
6. Proceed with automated extraction (→ "Preparing the Reagent Plate for automated extraction", p. 12).

10 Preparing the Reagent Plate for automated extraction

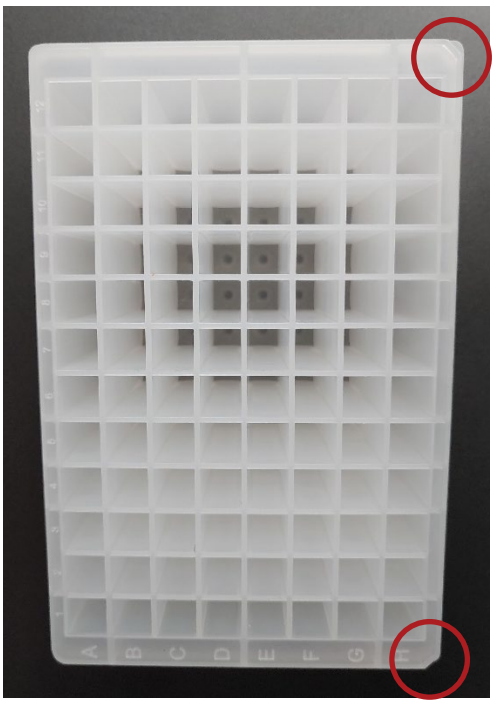
10.1 Prefilling of the Reagent Plate

NOTE

The Deep Well Plates have to be filled manually prior 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).

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	500 µl
	7	Washing Solution E	600 µl
	8	Washing Solution B2	600 µl
	9	Washing Solution B2	600 µl
	10	Washing Solution B2	600 µl
	11	empty	---
	12	RNase-free Water	600 µl

10.2 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)!

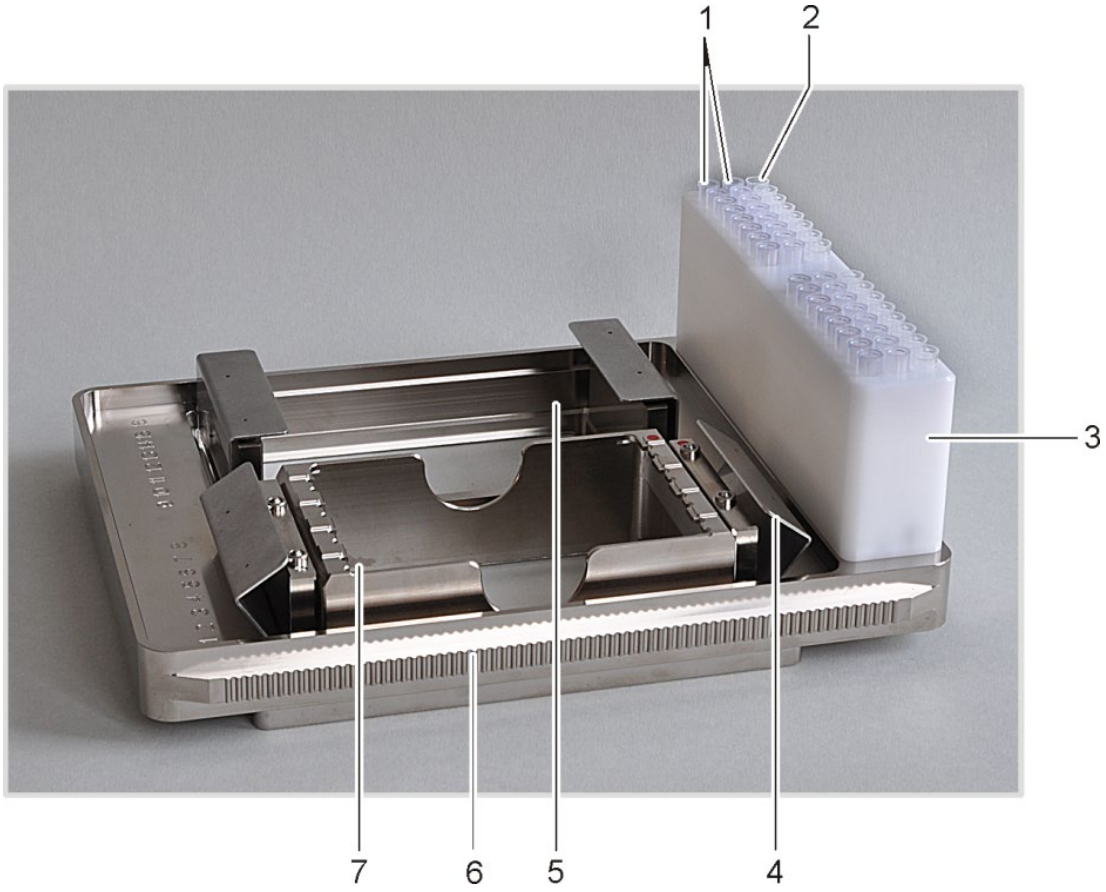
1. Transfer **50 µl** of **MAG Suspension** directly into the liquid of the **first cavity** of the Reagent Plate.
2. Transfer **400 µl** of **lysed sample** directly into the **third cavity** of Reagent Plate.

NOTE

The sample will be processed using the InnuPure C16 / C16 touch.
Please follow the instruction of chapter 11 on page 14.

11 Automated extraction using InnuPure C16 / C16 touch

11.1 Sample tray of InnuPure C16 / C16 touch



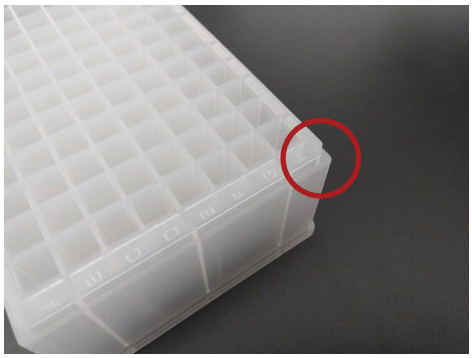
No. 1:	Filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Holding-down clamp
No. 5:	Sample block for Reagent Plates or adapter for Reagent Strips
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for Reagent Strips

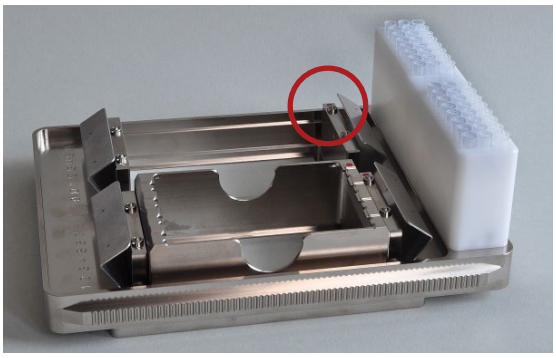
11.2 Preparing the sample tray of InnuPure C16 / C16 touch

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.

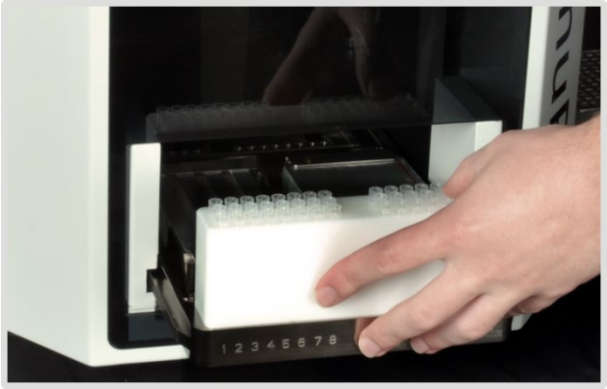
NOTE

Make sure that for every sample the tips and the elution vessel are in the corresponding positions of the tip block!

ATTENTION
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 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.



ATTENTION
Immediately let go of the sample tray as 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 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 **150–200 µl** and press [OK].

NOTE
It is possible to adjust the volume from 20 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 log file. 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 automatically.

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.

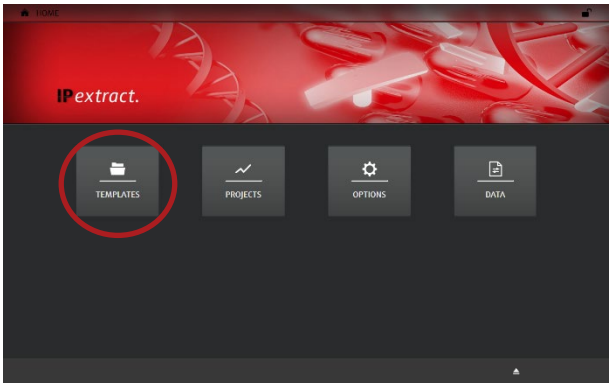
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA or RNA. Close the lids and store the DNA under proper conditions.

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

11.4 Starting the InnuPure C16 touch

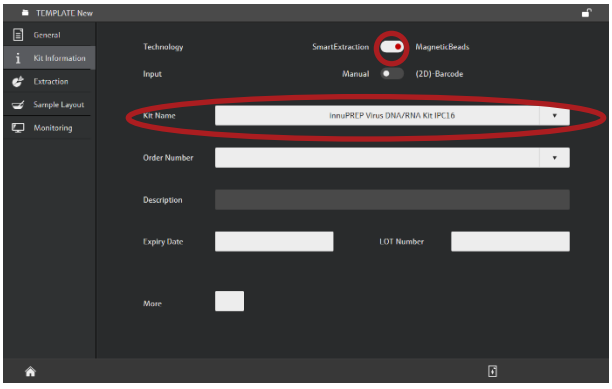
NOTE
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

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 the drop-down list “Kit Name”.



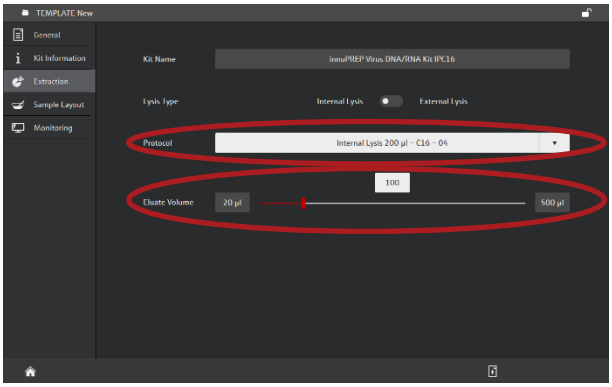
NOTE
“Kit Information” tab

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

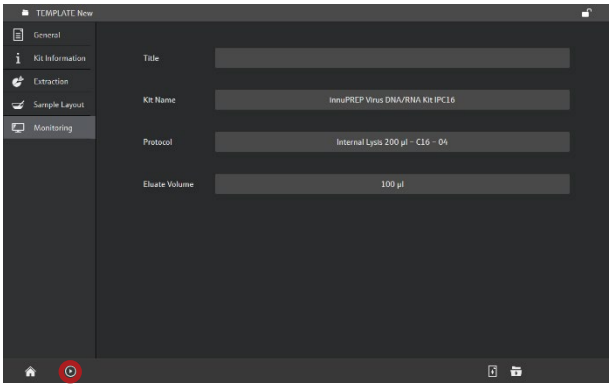
8. Adjust the “Eluate Volume“ using the slider or the text field.



NOTE
“Extraction” tab

The recommended elution volume is **150–200 µl**.

9. Choose the tab “Monitoring” and start the protocol by tapping the start button.



NOTE
“Monitoring” tab

10. Follow the instructions displayed on the tablet screen.

11. 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 to 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 or RNA. Close the lids and store the DNA under proper conditions.

NOTE
Store the DNA and RNA 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 DNA	
Insufficient lysis of starting material	Ensure to use the required volumes of **Proteinase K** and **Lysis Solution CBV**.
Eluate volume too high	Decrease the eluate volume. The suggested eluate volume is 150–200 µl. Please note that lowering the eluate volume will not increase the yield proportionally!
Inadequate extraction	Presence of inhibiting substances in the starting material. Please use the kit only for samples that match the requirements declared in “Product specifications”.

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