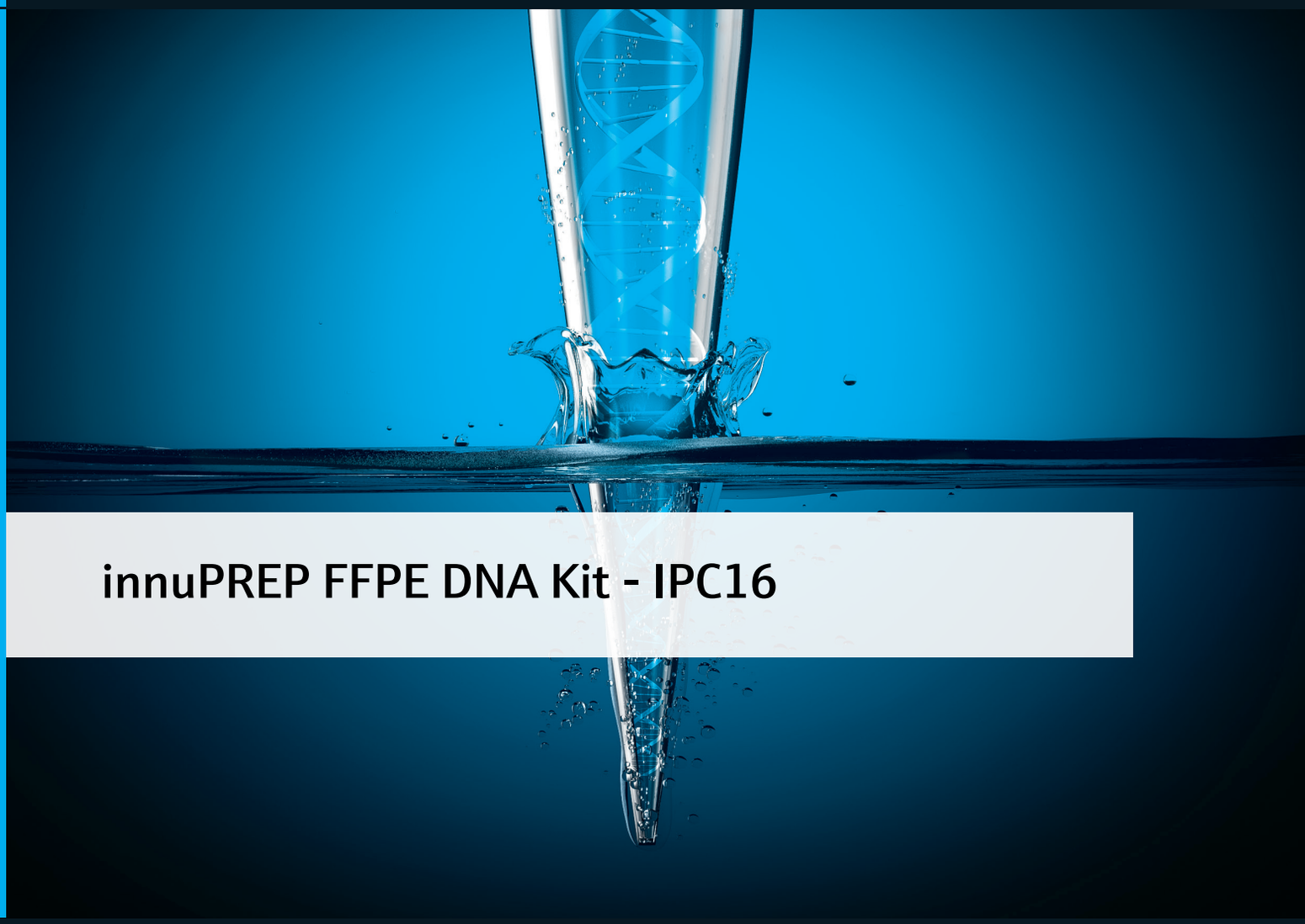
Instructions for Use
Life Science Kits & Assays



innuPREP FFPE DNA Kit - IPC16

Innuscreen GmbH

Order No.:

845-IPS-5916016	16 reactions
845-IPP-5916016	16 reactions
845-IPS-5916096	96 reactions
845-IPP-5916096	96 reactions
845-IPP-5916480	480 reactions

Publication No.: HB_IP-5916_e_220201

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 FFPE DNA Kit – IPC16 has been designed for automated isolation of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) samples using the InnuPure® C16 / C16 *touch*. The extraction procedure is based on a new patented chemistry.

The procedure starts with an external lysis step without the need for a deparaffinization step using toxic and hazardous components like octane or xylene. After the external lysis and incubation step the MAG Suspension F and the samples are 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 to surface-modified magnetic particles. After washing steps the nucleic acid is eluted from the magnetic particles with RNase-free water and is now ready to use. The extraction chemistry in combination with the InnuPure® C16 / C16 *touch* protocol is optimized to get maximum 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 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" p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully beforer to 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.

Store **MAG Suspension F** at 4 °C to 8 °C.

All other components of the innuPREP FFPE 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. 8.

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 FFPE 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. 10). 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-IP[S/P]-5916016	845-IP[S/P]-5916096	845-IPP-5916480
MAG Suspension F	0.25 ml	1.1 ml	5 × 1.1 ml
Lysis Solution BC	2 × 2 ml	2 × 12 ml	2 × 60 ml
Solution QPS	2 × 2 ml	25 ml	2 × 60 ml
Proteinase K	For 3 × 0.3 ml working solution	For 3 × 1.5 ml working solution	For 13 × 1.5 ml working solution
Reagent Strip I* (* Depending on order)	16 (pre-filled, sealed)	96 (pre-filled, sealed)	--
Reagent Plate I* (* Depending on order)	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 (Stripes)	2	12	5 × 12
Elution Strips	**2**	**12**	5 × 12
Manual	1	1	1

6.2 Components not included in the kit

- ddH_2O for dissolving **Proteinase K**
- 1.5 ml tubes
- 2.0 ml tubes, optional

7 Initial steps before starting

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

845-IPS-5916016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-IPP-5916016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-IPS-5916096	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-IPP-5916096	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-IPP-5916480	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Heat thermal mixer or water bath to 65 °C and 90 °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:
   - FFPE tissue samples
   - Approx. 2 × 5 µm; optional more starting material

2. Time for isolation:

   Preliminary steps: approx. 3.75 hours

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 amount and quality of starting material.

9 Protocol: Lysis of FFPE tissue samples

NOTE
The lysis of the starting material is a preliminary manual step. Heat two thermomixers or water baths to 65 °C and 90 °C, respectively.

1. Place the FFPE tissue sample (approx. 2x 5 µm or 1x 10 µm; optional more starting material) into a 2.0 ml reaction tube, close the cap and centrifuge the reaction tube at max. speed for 1 minute.
2. Open the reaction tube and add **200 µl Lysis Solution BC** and **40 µl Proteinase K** to the sample and mix vigorously by pulsed vortexing for 10 seconds. Centrifuge briefly to remove drops from the lid of the tube.

NOTE
The FFPE tissue sample has to be completely covered by **Lysis Solution BC**!

3. Incubate the reaction tube at 65 °C for 2.5 hours in a thermal mixer under continuous shaking at 1,000 rpm.

NOTE
Overnight incubation at 65 °C may increase the DNA yield. However, for most types of FFPE tissue sample the incubation at 65 °C for 2.5 hours is sufficient.

We recommend using a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation.

4. After the lysis step place the sample into a second thermal mixer pre-heated to 90 °C and incubate the sample for 1 hour.

NOTE
Do not place the sample into the thermal mixer, before the temperature of 90 °C is achieved!

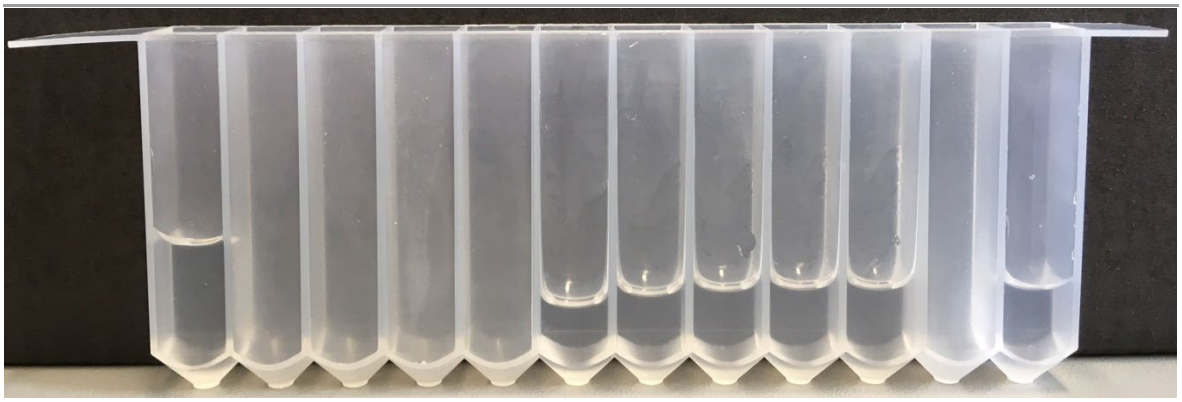
We recommend using a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation.

5. Open the reaction tube and add **200 µl Solution QPS** to the sample, mix vigorously by pulsed vortexing for 5 seconds.
6. Centrifuge the reaction tube at max. speed for 1 minute.
7. Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction", p. 17).

NOTE
If there is a thin film of paraffin above the sample, pierce this film carefully with pipette and carefully remove the sample.

10 Preparing Reagent Plate / Strip for automated extraction

10.1 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

10.2 Unpacking of Reagent Plate or Reagent Strip

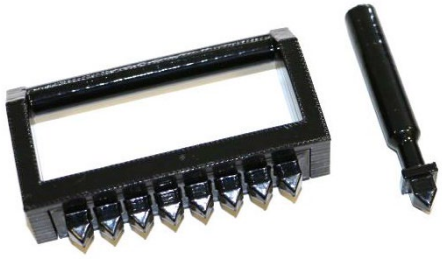
NOTE
According to transport regulations Reagent Reservoirs are wrapped into plastic bags only when transported by airplane.

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!

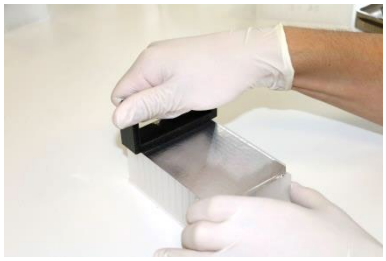
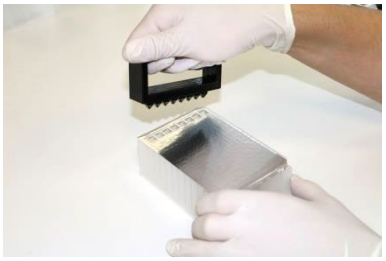
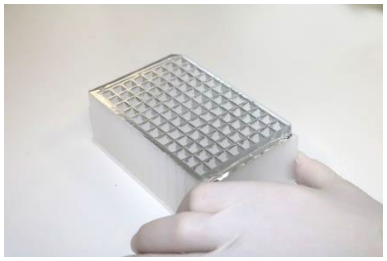


Reagent Plates or Strips are prefilled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (single piercer or 8fold piercer).

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

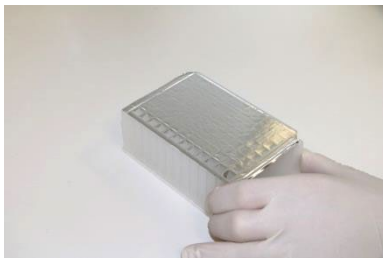
Open all cavities (one row per sample).

Using 8 samples in parallel

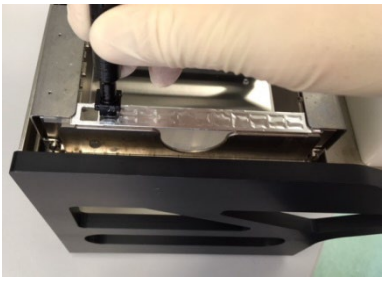
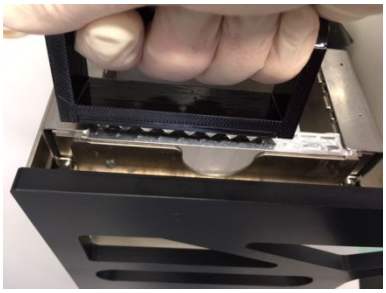
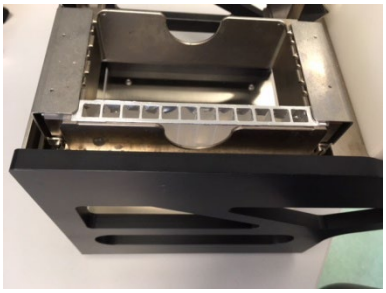




Using single samples





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 Reagent Plate or Reagent Strip

NOTE
It is important to mix the **MAG Suspension F** 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. 13).

1. Transfer **10 µl** of **MAG Suspension F** directly into the liquid of the **first cavity** of Reagent Plate or Reagent Strip.

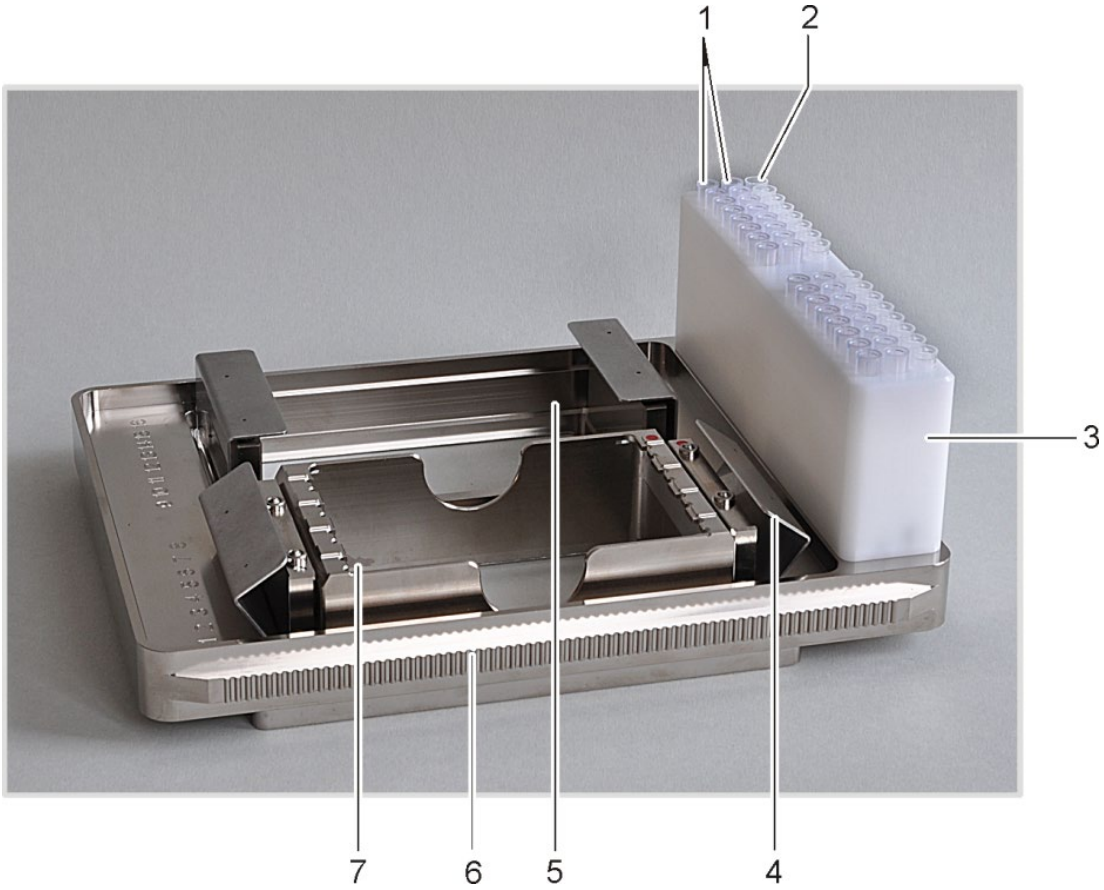
NOTE
It is important to mix the **MAG Suspension F** by vigorous shaking or vortexing before use (approx. 30 seconds).

2. Transfer **400 µl** of the **lysed sample** into the **third cavity** of Reagent Strip or Reagent Plate. Avoid carry-over of residual FFPE material!

NOTE
The sample will be processed using the InnuPure® C16 / C16 *touch*. Please follow the instructions of chapter 12 p. 18.

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

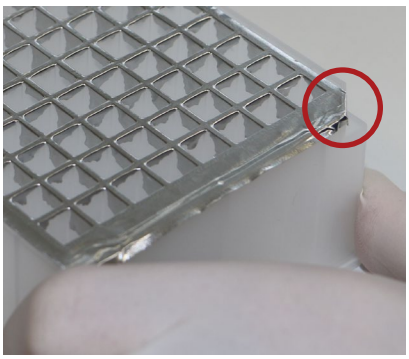
11.2 Preparing sample tray of InnuPure® C16 / C16 touch

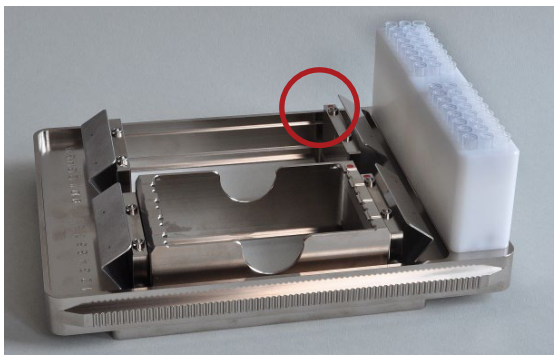
NOTE
The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more strips as number of samples!

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

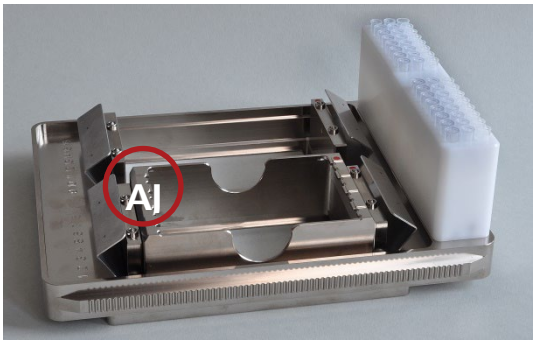
The notched corners of the Reagent Plate must point to the colored dot on the holder.





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

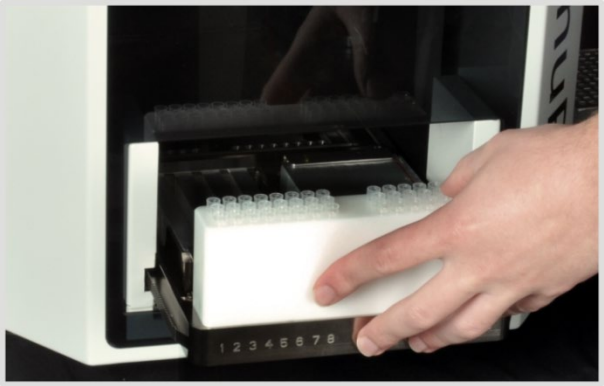
Especially with the Reagent Strips make sure that for every strip the tips and the elution vessel are in the corresponding positions in the tip block!

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 **100 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 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!

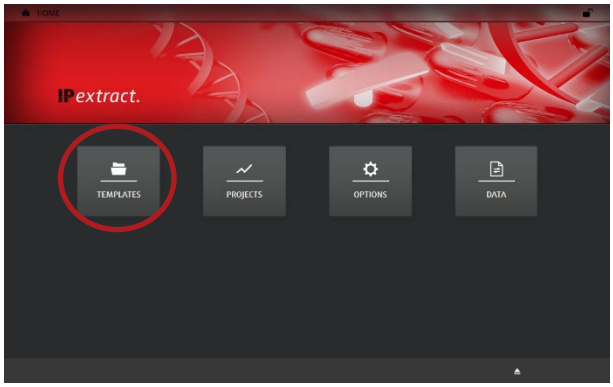
7. Remove the sample tray from the adapter of the InnuPure® C16 and place it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

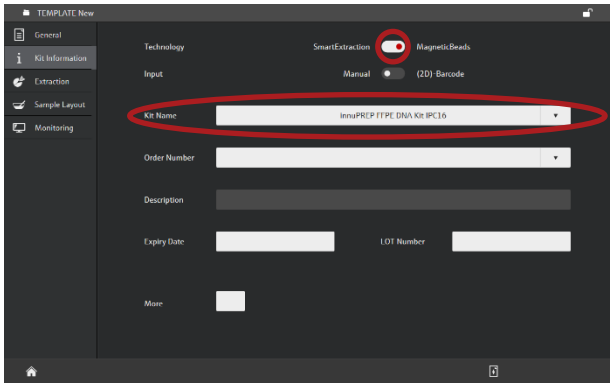
NOTE
The following instructions describe the necessary steps for the start of the InnuPure® C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure® C16 *touch*.

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”!

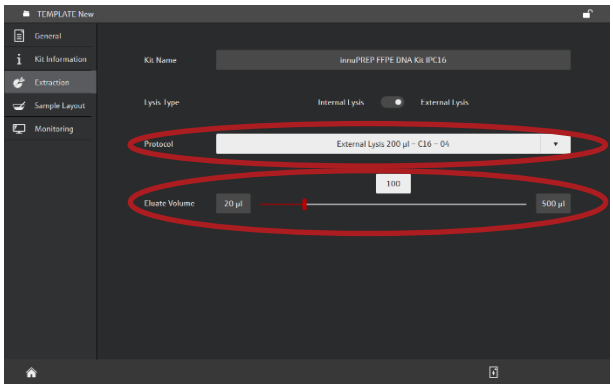


NOTE
“Kit Information” tab

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

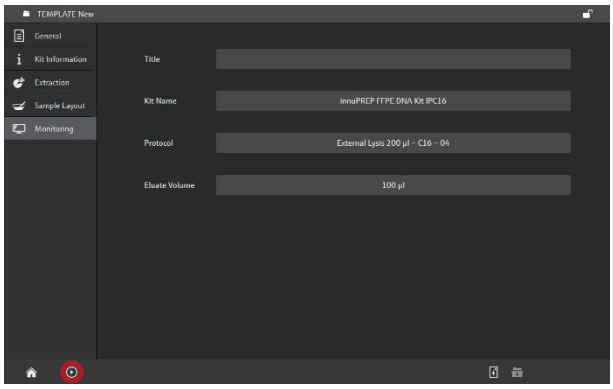
8. Adjust your desired “Eluate Volume“ using the slider or the text field.



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!

12 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 10 µl **MAG Suspension F** to **cavity 1** prior the extraction procedure. Ensure **Mag Suspension F** has mixed well before use.
Poor quality of extracted DNA	Avoid carryover of residual FFPE material when transferring lysed sample to cavity 3 of Reagent Plate/Strip.
Insufficient lysis of starting material	Perform lysis at 65 °C for at least 2.5 hours. Ensure to use the required volume of **Proteinase K** and **Lysis Solution BC**.
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!

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