

Instructions for Use

Life Science Kits & Assays



innuPREP FFPE DNA Kit – IPC16

Order No.:

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

IPS = Kit contains prefilled reagent strips for processing individual samples

IPP = Kit contains prefilled reagent plates for running 8 samples in parallel

Note: Prefilled reagent strips and reagent plates can be used in parallel in the InnuPure® C16.

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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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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 prior to 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

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

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

The following systematic approach is introduced in the manual:

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

2 Safety precautions

NOTE

Read through this chapter carefully prior 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. Analytik Jena AG 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, please ask for the Safety Data Sheets (SDS).

3 Storage conditions

Store lyophilized **Proteinase K** at 4 °C to 8 °C! Divide dissolved **Proteinase K** into aliquots and storage at -22 °C to -18 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!

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 Function testing and technical assistance

The Analytik Jena AG 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 Analytik Jena AG products, please do not hesitate to contact us.

For technical support or further information in Germany please dial +49 36 41 / 77 94 00. 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. 14). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols than those described below. Analytik Jena AG 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 Analytik Jena AG 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

IMPORTANT

Store lyophilized **Proteinase K** at 4 °C to 8 °C. Divide dissolved **Proteinase K** into aliquots. Storage at -22 °C to -18 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!

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



STORAGE CONDITIONS

All other components are stored at room temperature.

	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 of order)	16 (pre-filled, sealed)	96 (pre-filled, sealed)	--
Reagent Plate I* (* Depending of 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
Initial steps	Proteinase K Dissolve by addition of 0.3 ml of ddH ₂ O, mix thoroughly and store as described above.	Proteinase K Dissolve by addition of 1.5 ml of ddH ₂ O, mix thoroughly and store as described above.	




COMPONENTS NOT INCLUDED IN THE KIT

- 1.5 ml tubes
- 2.0 ml tubes, optional
- ddH₂O for dissolving **Proteinase K**

7 Recommended steps before starting

- Ensure that the **Proteinase K** has been prepared according to the instruction (→ “Kit components”, p. 8).
- 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 GHS Classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Reagent Plate/Strip I	Propan-2-ol 50–100 %		225, 315, 318, 336, 411	101, 102, 103, 210, 303+361+353, 305+351+338, 310, 405, 501
	Polyeth- ylene glycol octylphenol ether 25–50 %		Danger	
	Ethanol 50–100 %			
Proteinase K	Proteinase, engyodonti- um album		315, 317, 319, 334, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501

8.1 Hazard phrases

- 225 Highly flammable liquid and vapor.
- 315 Causes skin irritation.
- 317 May cause an allergic skin reaction.
- 318 Causes serious eye damage.
- 319 Causes serious eye irritation.
- 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
- 335 May cause respiratory irritation.
- 336 May cause drowsiness or dizziness.
- 411 Toxic to aquatic life with long lasting effects.

8.2 Precaution phrases

- 101 If medical advice is needed, have product container or label at hand.
- 102 Keep out of reach of children.
- 103 Read label before use.
- 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
- 261 Avoid breathing dust/fume/gas/mist/vapors/spray.
- 280 Wear protective gloves/protective clothing/ eye protection/face protection.
- 310 Immediately call a POISON CENTER/doctor.
- 405 Store locked up.
- 501 Dispose of contents/container in accordance with local/regional/national/international regulations.
- 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
- 303+361+ IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
353
- 305+351+ IF IN EYES: Rinse cautiously with water for several minutes.
338 Remove contact lenses, if present and easy to do. Continue rinsing.

9 Product specifications

1. Starting material:
 - FFPE tissue samples
 - Approx. $2 \times 5 \mu\text{m}$; optional more starting material

2. Time for isolation:

Preliminary steps: approx. 3.75 hours

Extraction protocol	Protocol on In-nuPure®C16 / C16 touch	Time In-nuPure®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.

10 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.

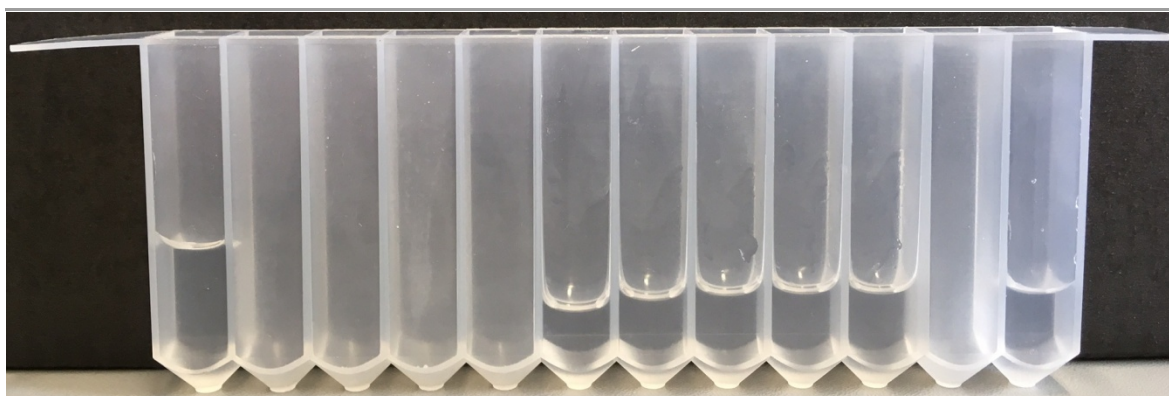
5. Open the reaction tube and add **200 µl Solution QPS** to the sample, mix vigorously by pulsed vortexing for 5 seconds.
6. Centrifuge the reaction tube at max. speed for 1 minute.
7. Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction", p. 17).

NOTE

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

11 Preparing Reagent Plate / Strip for automated extraction

11.1 General filling scheme of reagent reservoir



Cavity 1:	RNase-free Water	Cavity 7:	Washing Solution
Cavity 2:	Empty	Cavity 8:	Washing Solution
Cavity 3:	Empty	Cavity 9:	Washing Solution
Cavity 4:	Empty	Cavity 10:	Washing Solution
Cavity 5:	Empty	Cavity 11:	Empty
Cavity 6:	Binding Solution	Cavity 12:	Elution Buffer

11.2 Unpacking of Reagent Plate or Reagent Strip

NOTE

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



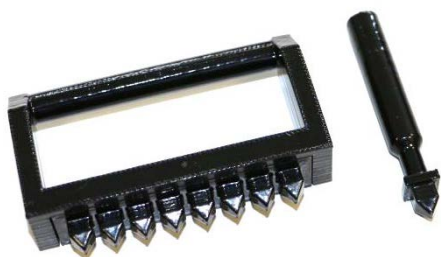
Reagent Plates or Reagent Strips are delivered wrapped into plastic bags for transport protection.

Carefully open the overpack of Reagent Plates or Strips by using scissors.

11.3 Piercing of sealing foil of Reagent Plate or Reagent Strip

NOTE

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

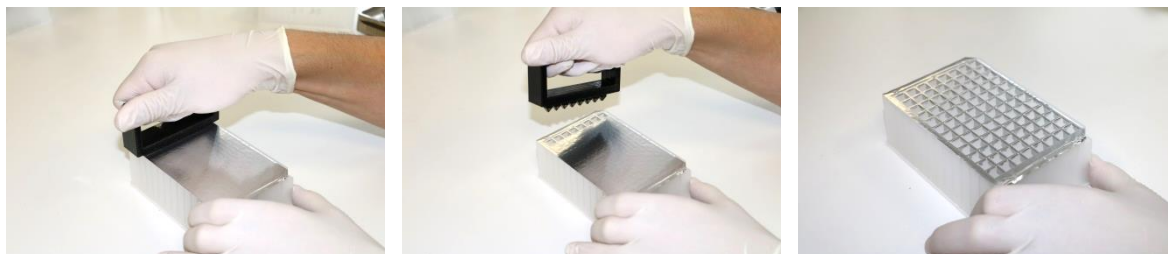


Reagent Plates or Strips are prefilled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (single piercer or 8fold piercer).

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



Preparing Reagent Plate / Strip for automated extraction

Using Reagent Strips



IMPORTANT

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

11.4 Loading the sample to 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. 17).

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).

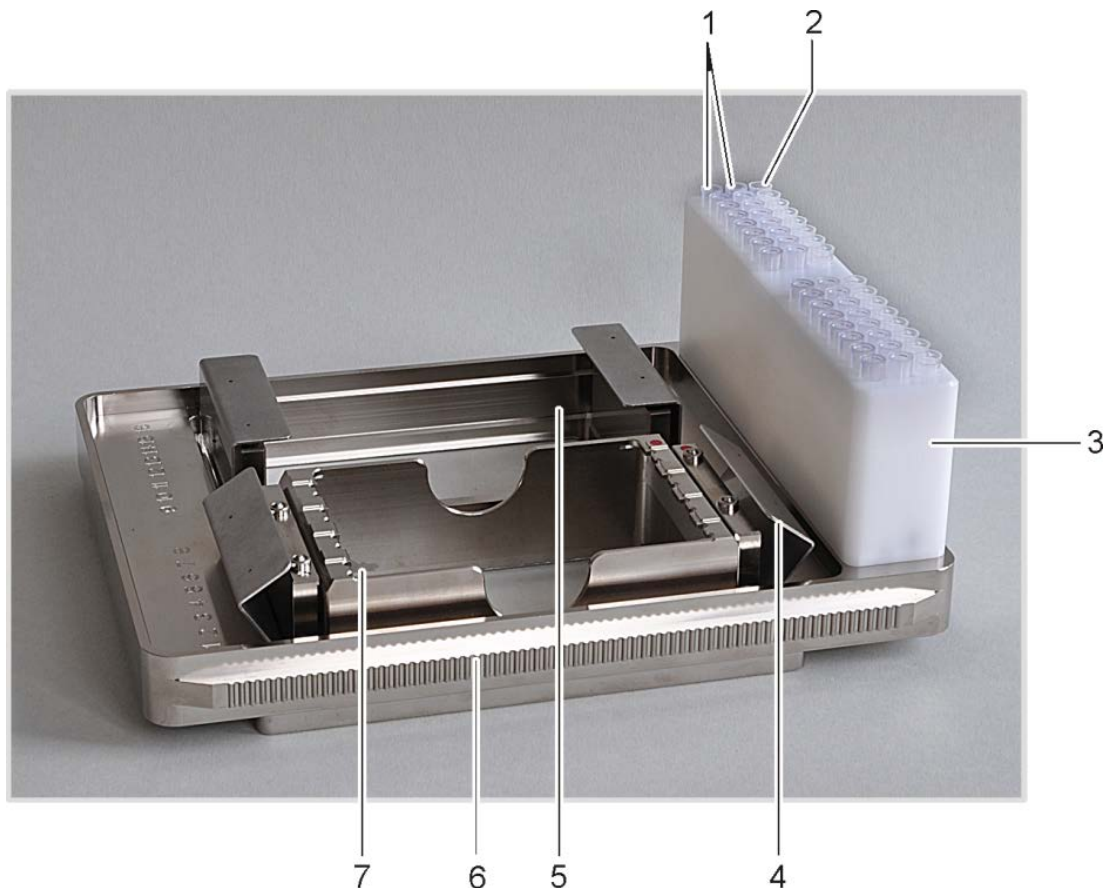
2. Transfer **400 µl** of the **lysed sample** into the **third cavity** of Reagent Strip or Reagent Plate. Avoid carry-over of residual FFPE material!
-

NOTE

The sample will be processed using the InnuPure® C16 / C16 *touch*. Please follow the instructions of chapter 12 p. 22.

12 Automated extraction using InnuPure® C16 / C16 touch

12.1 Sample tray of InnuPure® C16 / C16 touch



No. 1: Filter tips

No. 2: Elution vessels for purified samples

No. 3: Tip block

No. 4: Holding-down clamp

No. 5: Sample block for reagent plates or adapter for reagent strips

No. 6: Serrated guide rail (C16 *touch*: non-serrated)

No. 7: Adapter for reagent strips

12.2 Preparing sample tray of InnuPure® C16 / C16 touch

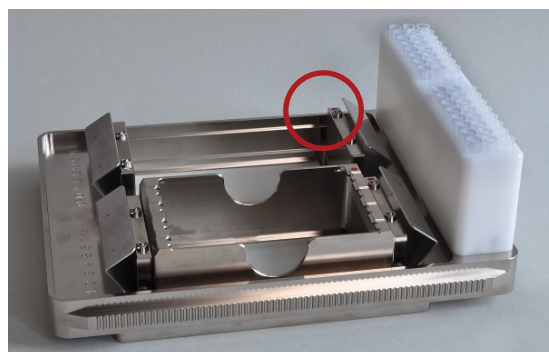
NOTE

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

1. Place the InnuPure® C16 / C16 *touch* sample tray into the priming station and fold the holding-down clamp at the sample tray upwards!
2. Place the Reagent Plate or an adapter with Reagent Strips into the holder of the sample tray. Using Reagent Plates, the notched corner of the Reagent Plate has to align with the colored dot at the holder. Using adapters and Reagent Strips, the colored dot of the adapter has to align with the colored dot at the holder and Reagent Strips have to be inserted in a way that the "AJ" labels are arranged at the side of the adapter which is more distant from the tip block.

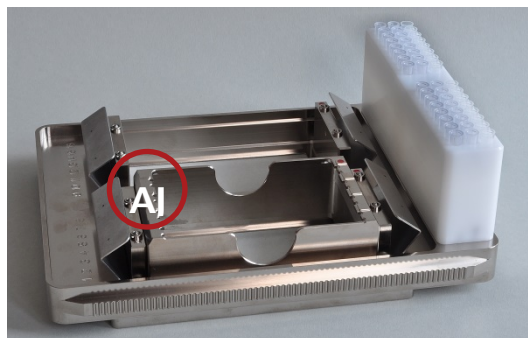
Reagent Plate

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



Reagent Strips

Place the strips into the adapter. The long tab marked with the label "AJ" must point to the side of the adapter which is more distant from the tip block.



CAUTION

Both holders have to be equipped with a Reagent Plate or Reagent Strips. If applicable use an empty or dummy plate for the respective holder.

3. Fold down the holding-down clamp to prevent the Reagent Plates and Reagent Strips to be pulled out of the holder during the extraction process.
4. For each extracted sample place two filter tips in the smaller drill holes of the tip block.
5. Place the Elution Tubes into the wider drill hole at the edge of the tip block. Empty sample positions do not need to be filled.

NOTE

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

IMPORTANT NOTE

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

12.3 Starting the InnuPure® C16

1. Switch on the InnuPure® C16 and wait for the device initialization to complete, which is signaled by a beeping sound.
2. Move the loaded sample tray with the Reagent Strips or Reagent Plates forward into the sample tray adapter on the front of the InnuPure® C16. The serrated rails at the side of the sample tray must protrude into the grooves of the adapter. After pressing lightly against the tip block the sample tray is automatically pulled into the device.



IMPORTANT – CAUTION

Risk of crushing

Immediately let go of the sample tray once it is being pulled in. Otherwise there is a risk of your hand being crushed.

3. After pressing [Select Protocol] choose an appropriate extraction protocol on InnuPure® C16 and press [Start]:

Extraction procedure	Protocol on 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

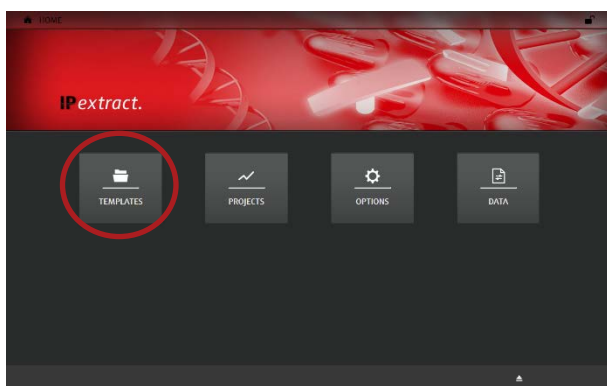
Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

12.4 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*.

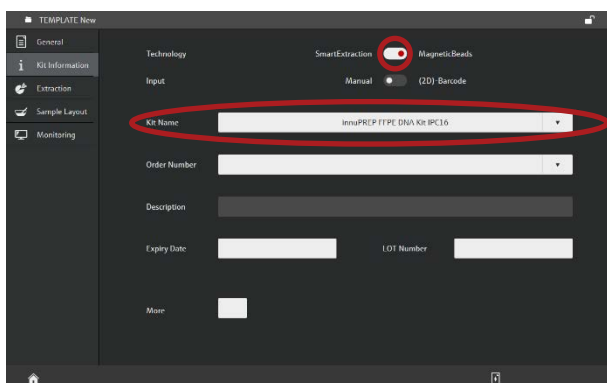
1. Switch on the InnuPure® C16 *touch* and the tablet computer. Wait until the home screen of IPextract is displayed on the tablet screen.



NOTE

Home screen of IPextract

2. Choose [TEMPLATES]→[New Template]→[Kit-based].
3. Enter optional information in the tab “General”.
4. Choose the tab “Kit Information” and switch the “Technology” to “MagneticBeads”!
5. Choose your desired kit from “Kit Name”!



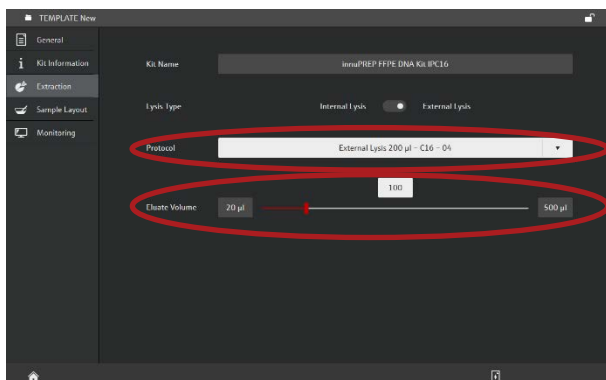
NOTE

“Kit Information” tab

6. Enter optional information in the tab "Kit Information"
7. Choose the tab "Extraction" and choose the desired "Protocol"

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

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

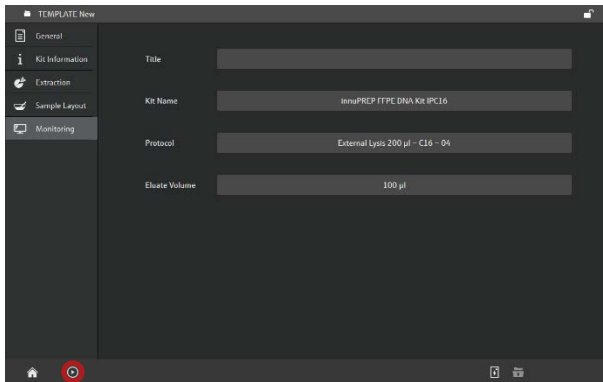


NOTE

"Extraction" tab

The recommended elution volume is 100 µl.

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



NOTE
“Monitoring” tab

10. Follow the instructions displayed on the tablet screen.
11. Completion of the protocol is indicated by a message on the tablet screen. Follow the instructions on the screen to remove the sample tray from the device.
12. The Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

NOTE

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

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted genomic DNA	
No extracted DNA	No magnetic beads added to cavity 1. Please add 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!

14 Related Products

Name	Amount	Order No.
Nucleic acid purification		
innuPREP Proteinase K		
	6 mg	845-CH-0010006
	30 mg	845-CH-0010030
Automated nucleic acid purification		
smart Blood DNA Midi prep (a)		
	16 rxn (Strips)	845-ASS-1208016
	96 rxn (Strips)	845-ASS-1208096
	16 rxn (Plates)	845-ASP-1208016
	96 rxn (Plates)	845-ASP-1208096
smart DNA prep (a)		
	16 rxn (Strips)	845-ASS-2008016
	96 rxn (Strips)	845-ASS-2008096
	16 rxn (Plates)	845-ASP-2008016
	96 rxn (Plates)	845-ASP-2008096
innuPREP Virus DNA/RNA Kit – IPC16		
	16 rxn (Strips)	845-IPS-5016016
	96 rxn (Strips)	845-IPS-5016096
	16 rxn (Plates)	845-IPP-5016016
	96 rxn (Plates)	845-IPP-5016096
	480 rxn (Plates)	845-IPP-5016480
Products for PCR & Electrophoresis		
innuTaq DNA Polymerase (5 U/μl)		
	500 U	845-EZ-1000500
50x inNucleotide Mix (1.5 mM)		
	2x 0.5 ml	845-AS-9000100
innuDRY Standard PCR Master Mix		
	100 rxn	845-AS-2100100
	200 rxn	845-AS-2100200
innuDRY qPCR MasterMix Probe		
	100 rxn	845-AS-1900100
	200 rxn	845-AS-1900200

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Pictures: Analytik Jena AG
Subject to changes in design and scope of delivery as well as further technical development!