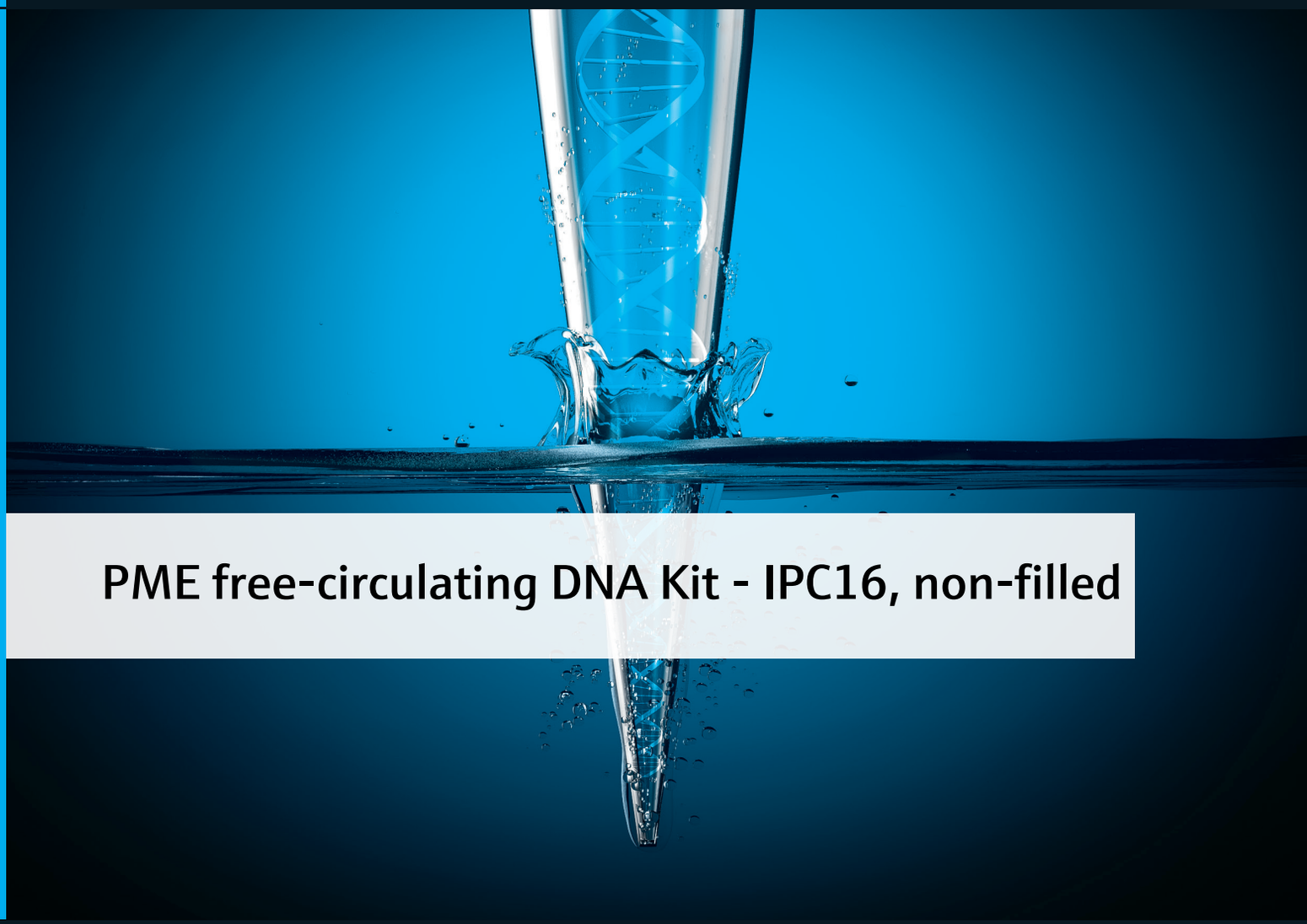
Instructions for Use
Life Science Kits & Assays



PME free-circulating DNA Kit - IPC16, non-filled

iST Innuscreen GmbH

Order No.:

845-PPP-6116016	16 reactions
845-PPP-6116096	96 reactions
845-PPP-6116480	480 reactions

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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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Contents

1 Introduction

1.1 Intended use

Free-circulating DNA in serum, plasma or in urine is very important as diagnostic target. The content of free-circulating DNA is usually very low and varies among different individuals. Further, the free-circulating DNA is present as short fragments, usually smaller than 1000 bp. Therefore, the extraction of cell-free-circulating DNA is difficult. Conventional kits use standard nucleic acid extraction procedures based on sample lysis, binding the nucleic acids on a solid material, washing and elution of nucleic acids. Because of the high sample volume the procedures are very time- and work-consuming and need a lot of reagents.

The **PME free-circulating DNA Kit - IPC16, non-filled** is based on a new technology, called: PME – Polymer Mediated Enrichment. The procedure does not start with sample lysis, like commonly used methods or kits. The first step is capturing the free-circulating DNA with a special polymer. Subsequently the captured free-circulating DNA is dissolved in a special buffer and then the DNA is extracted automatically using the InnuPure® C16 / C16 *touch*. 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 free-circulating DNA on surface-modified magnetic particles. After washing steps, the nucleic acid is eluted from the magnetic particles with a low volume of RNase-free water and is now ready to use. The extraction chemistry in combination with the InnuPure® C16 / C16 *touch* protocol is optimized to obtain maximum yield and quality. The whole automatic procedure for the isolation of free-circulating DNA from 1 ml of sample volume takes approx. 66 minutes and from 2 ml to 5 ml serum or plasma or 5 ml to 10 ml urine approx. 70 minutes.

The kit contains a Carrier RNA. Addition of Carrier RNA is recommended if an extremely low amount of free-circulating DNA is expected. In this case, the addition of Carrier RNA can increase the final yield. Using real-time

PCR as a downstream application, this has shown a benefit of 0.5 – 1 Ct-value. In all other cases the addition of Carrier RNA is not necessary.

The kit works with 1 ml to 5 ml serum, plasma or urine sample. The extracted free-circulating DNA is suitable for downstream applications like PCR, real-time PCR, bisulfite conversion or any kind of enzymatic reaction.

The detection limit for certain free-circulating DNA depends on the individual procedures, for example in-house PCR or commercially used detection assays.

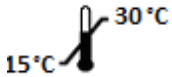
Please note that in case of using the Carrier RNA the eluates contain free-circulating DNA and Carrier RNA. In this case, the extracted nucleic acids are not suitable for some downstream applications like Next Generating Sequencing (NGS) or the quantification of nucleic acids (isolated with this kit) by photometric or fluorometric methods. It is recommended to quantify extracted DNA with other methods like specific quantitative PCR or real-time PCR, or to not use the Carrier RNA. Furthermore, Carrier RNA may inhibit PCR reactions. Thus, the amount of added Carrier RNA has to be carefully optimized depending on the individual PCR system used.

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** 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 and the kit" p. 4).
- 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.

Upon arrival, store lyophilized **Proteinase K** and **Enrichment Reagent VCR-1** and **MAG Suspension** at 4 °C to 8 °C.

Store lyophilized **Carrier RNA** at -22 °C to -18 °C.

It is recommended to divide dissolved Carrier RNA stock solution into aliquots for storage at -22 °C to -18 °C. Do not freeze and thaw Carrier RNA stock solution more than 3 times.

All other components of the **PME free-circulating 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 dissolve these precipitates by careful warming.

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced 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 **PME free-circulating 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 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

	Σ 16	Σ 96	Σ 480
REF	845-PPP-6116016	845-PPP-6116096	845-PPP-6116480
Enrichment Reagent VCR-1	2 x 1.2 ml	8 x 1.2 ml	44 x 1.2 ml
Enrichment Reagent VCR-2	10 ml	2 x 32 ml	5 x 60 ml
Lysis Solution SE	15 ml	60 ml	4 x 80 ml
Carrier RNA	For 1 ml working solution	For 1 ml working solution	For 6 x 1 ml working solution
RNase-free Water	1 x 2.0 ml	1 x 2.0 ml	6 x 2.0 ml
Proteinase K	For 2 × 0.3 ml working solution	For 2 × 1.5 ml working solution	For 11 × 1.5 ml working solution
MAG Suspension	1 ml	5.5 ml	3 x 9 ml
RNase-free Water	30 ml	2 x 80 ml	2 x 400 ml
Binding Solution SBS	15 ml	90 ml	2 x 240 ml
Washing Solution HS (conc.)	6 ml	40 ml	2 x 80 ml
Washing Solution LS (conc.)	5 ml	25 ml	130 ml
Deep Well Plate (2.0 ml)	2	12	60
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	5 x 12
Manual	1	1	1

6.1 Components not included in the kit

- ddH_2O for dissolving **Proteinase K** and working steps of protocol 1 and protocol 2
- 96–99.8 % ethanol (molecular biology grade, non-denatured)
- 1.5 ml reaction tubes
- 15 ml reaction tubes

7 Initial steps before starting

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

845-PPP-6116016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-PPP-6116096	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-PPP-6116480	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of absolute ethanol to **Washing Solution HS** and mix thoroughly. Always keep the bottle firmly closed!

845-PPP-6116016	Add 6 ml of **96-99.8 % ethanol** to 6 ml **Washing Solution HS (conc.)**
845-PPP-6116096	Add 40 ml of **96-99.8 % ethanol** to 40 ml **Washing Solution HS (conc.)**
845-PPP-6116480	Add 80 ml of **96-99.8 % ethanol** to 80 ml **Washing Solution HS (conc.)**

- Add the indicated amount of absolute ethanol to **Washing Solution LS** and mix thoroughly. Always keep the bottle firmly closed!

845-PPP-6116016	Add 20 ml of **96-99.8 % ethanol** to 5 ml **Washing Solution LS (conc.)**
845-PPP-6116096	Add 100 ml of **96-99.8 % ethanol** to 25 ml **Washing Solution LS (conc.)**

845-PPP-6116480	Add 520 ml of **96-99.8 % ethanol** to 130 ml **Washing Solution LS (conc.)**

- Add 1 ml RNase-free Water to each vial of lyophilized **Carrier RNA,** mix thoroughly by pipetting up and down and store as described above.
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

8 Product specifications

1. Starting material:
   - Serum, plasma, cell culture supernatants or mediums and other cell-free body fluids from 1 ml up to 5 ml
   - Urine from 5 ml to 10 ml

2. Time for isolation:
   - Enrichment procedure

     1 ml protocol approx: 10 minutes

     5 ml protocol approx: 30 minutes

   - Automated extraction

Extraction protocol	Protocol on InnuPure®C16 / C16 touch	Time InnuPure®C16 / C16 touch	Elution volumes
PME_1ml_C16_04/ PME 1 ml – 05	200 µl	70 / 69 min	20–500 µl
PME_5ml_C16_04/ PME 5 ml – 05	200 µl	79 / 77 min	20–500 µl

9 General procedure of enrichment and isolation of free-circulating DNA

	up to 1 ml serum/plasma or other cell-free body fluids (except urine)	2 ml up to 5 ml serum/plasma or other cell-free body fluids or 5 ml up to 10 ml urine
Enrichment of free-circulating DNA		 **Capturing of cell-free DNA in the polymer** **Spin down of the Polymer/DNA complex**

- Lysis of the Polymer/DNA complex and sample preparation of free-circulating DNA runs automatically on the InnuPure®C16 / C16 *touch*.

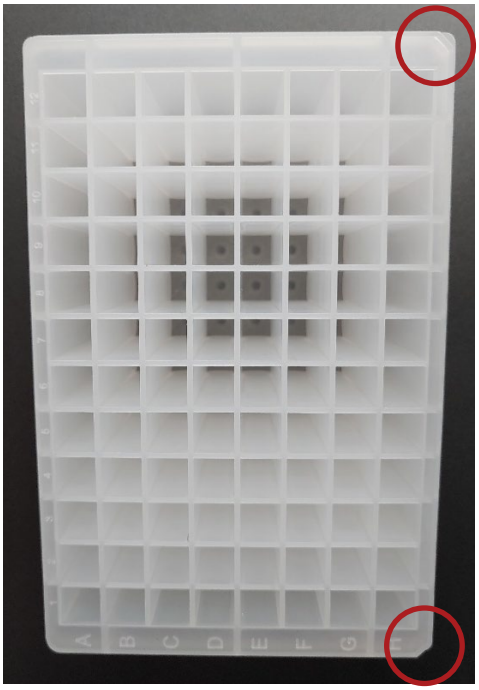
10 Preparing Reagent Plates for automated extraction

NOTE

The Deep Well Plates have to be filled manually prior to 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 volumes 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) and also add **MAG Suspension**, **Sample** and **Proteinase K** as described in the chapter “Loading the sample to InnuPure® C16 / C16 *touch*” (p. 15).

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	800 µl
	7	Washing Solution HS	600 µl
	8	Washing Solution LS	600 µl
	9	Washing Solution LS	600 µl
	10	empty	---
	11	empty	---
	12	RNase-free Water	600 µl

11 Protocols

11.1 Protocol 1: Isolation of free-circulating DNA from serum, plasma, or other cell-free body fluids (except urine) up to 1 ml

NOTE
The enrichment of free-circulating DNA is a preliminary manual processing step.

1. Add **30 µl** of Enrichment Reagent **VCR-1** and the sample into a 1.5 ml reaction tube and vortex shortly. Add **150 µl** of Enrichment Reagent **VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 1 minute.
2. Centrifuge at max. speed for 3 minutes, open the tube and remove the supernatant carefully as much as possible.
3. Add **1 ml ddH_2O** and centrifuge at max. speed for 3 minutes, open the tube and remove the supernatant carefully as much as possible.

NOTE
Don’t remove the pellet; it will be processed like the following steps!

4. Add **400 µl Lysis Solution SE** to the reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times. Avoid thereby the formation of air bubbles!

NOTE
Optional add 10 µl Carrier RNA to the sample after adding Lysis Solution SE. See "Intended use" p. 3 if Carrier RNA is necessary to add or not!

5. Proceed with automated extraction (→ "Loading the sample to InnuPure® C16 / C16 *touch*", p. 15).

11.2 Protocol 2: Isolation of free-circulating DNA from serum, plasma, or other cell-free body fluids (except urine) of 2 ml up to 5 ml and from urine sample from 5 ml up to 10 ml

NOTE
Urine samples content cellular materials and cellular nucleic acids. In order to enrich only free-circulating DNA from the urine sample it is recommended to centrifuge the urine sample at max. speed (e.g. 16,000 x g) and work subsequently only with the supernatant.

1. Add **100 µl** of Enrichment Reagent **VCR-1** and the sample into a 15 ml reaction tube and vortex shortly. Add **600 µl** of Enrichment Reagent **VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 10 minutes.
2. Centrifuge the tubes at least at 4,500 x g (~5,400 rpm) for 10 minutes, open the tube and remove the supernatant carefully as much as possible.
3. Add **5 ml ddH_2O** to the tube and centrifuge at least at 4,500 x g (~5,400 rpm) 5 minutes, open the tube and remove the supernatant carefully as much as possible.

NOTE
Don’t remove the pellet; it will be processed like the following steps!

4. Add **600 µl Lysis Solution SE** to the 15 ml reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times. Avoid thereby the formation of air bubbles!

NOTE
Optional add 10 µl Carrier RNA to the sample after adding Lysis Solution SE. See "Intended use" p. 3 if Carrier RNA is necessary to add or not!

5. Proceed with automated extraction (→ "Loading the sample to InnuPure® C16 / C16 *touch*", p. 15).

11.3 Loading the sample to InnuPure® C16 / C16 touch

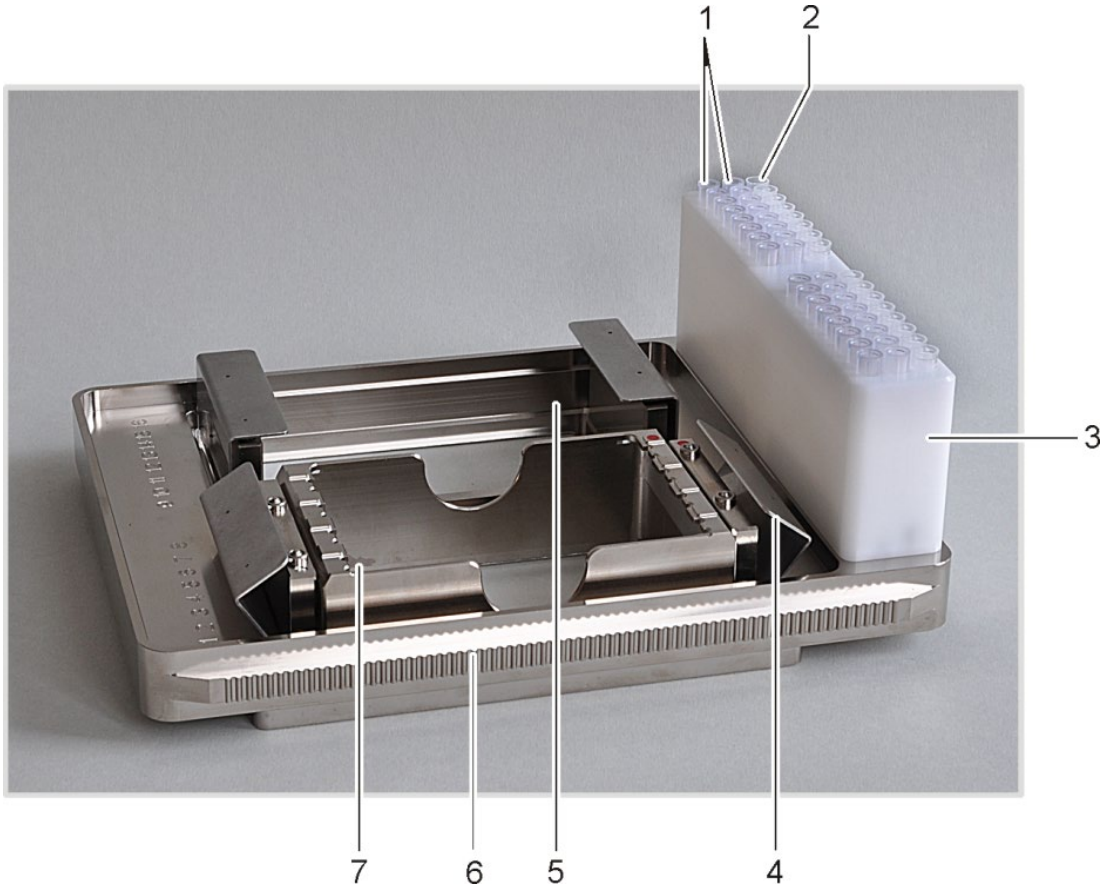
NOTE
It is important to mix the MAG Suspension by vigorous shaking or vortexing before use (approx. 30 seconds!

1. Transfer **50 µl** of **MAG Suspension** directly to the liquid of the **first cavity** of Reagent Plate.
2. Transfer **the whole lysed sample** and **25 µl Proteinase K** into the **third cavity** of Reagent Strip or Reagent Plate. Avoid carry-over of solid material!

NOTE
The sample will be processed using the InnuPure® C16 / C16 *touch*. Please follow the instructions of chapter 12 ("Automated extraction using InnuPure® C16 / C16 *touch*", p. 16.

12 Automated extraction using InnuPure® C16 / C16 touch

12.1 Sample tray of InnuPure® C16 / C16 touch



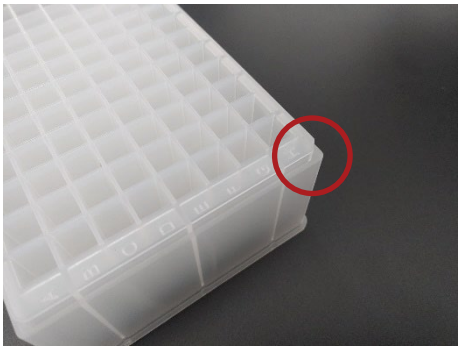
No. 1:	Filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Holding-down clamp
No. 5:	Sample block for reagent plates or adapter for reagent strips
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for reagent strips

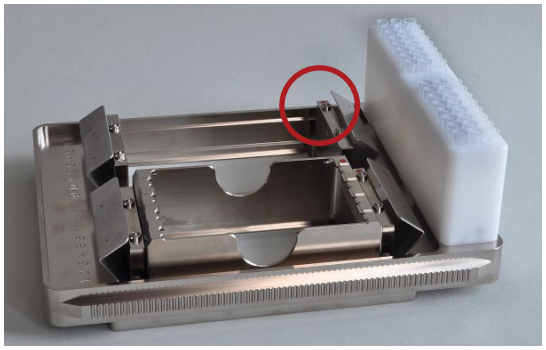
12.2 Preparing sample tray of InnuPure® C16 / C16 touch

1. Place the InnuPure® C16 / C16 *touch* sample tray into the priming station and open the holding-down clamp 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 at the holder.

Reagent Plate

The notched corners 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 clamp 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 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

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

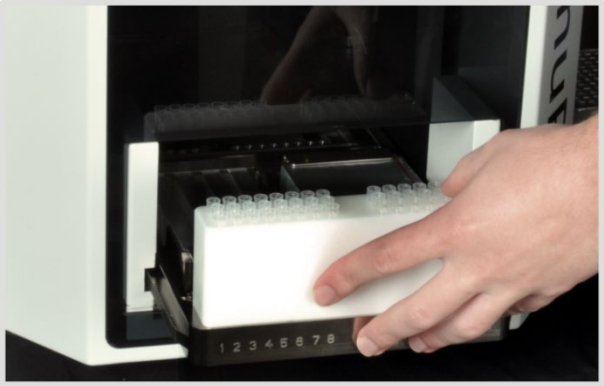
IMPORTANT NOTE

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
Protocol 1	PME_1ml_C16_04
Protocol 2	PME_5ml_C16_04

4. Enter the recommended **elution Volume** of **50 µl** and press [OK].

NOTE
It is possible to adjust the volume values from20 µl to 500 µl.

5. If needed, choose log-file and enter sample ID's, press [OK] or [CANCEL].

NOTE
It is possible to enter sample ID's and to create a run logfile. Find more detailed information how to start an extraction protocol using InnuPure® C16 on page 37 of the user manual "6.3.5 Using the sample setup tool"!

6. After completion of the protocol press [NEXT] and the sample tray is then automatically moved out of the device.

NOTE
The chosen protocol is performed by the device and after the protocol is finished, the tray with the purified samples will be moved out after pressing [NEXT] and the message 'Program finished' is shown on the screen of the device!

7. Remove the sample tray from the adapter of the InnuPure® C16 and place it back into the priming station.

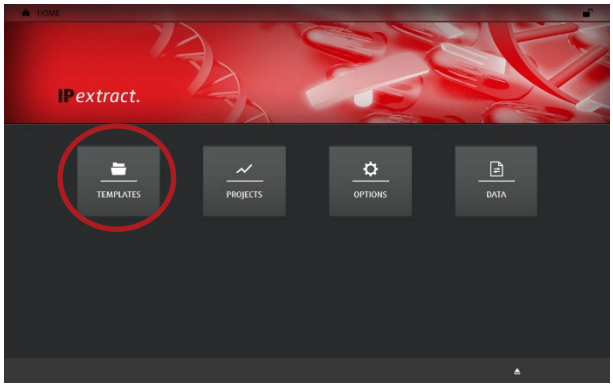
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

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*.

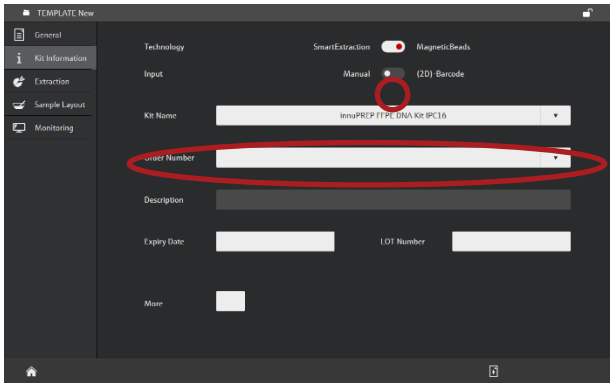
1. Switch on the InnuPure® C16 *touch* and the tablet computer. Wait until the home screen of IP*extract* is displayed on the tablet screen.



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”!

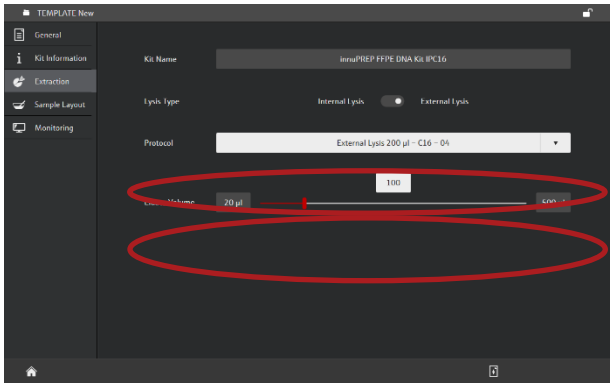


NOTE
“Kit Information” tab

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
Protocol 1	PME 1 ml – 05
Protocol 2	PME 5 ml – 05

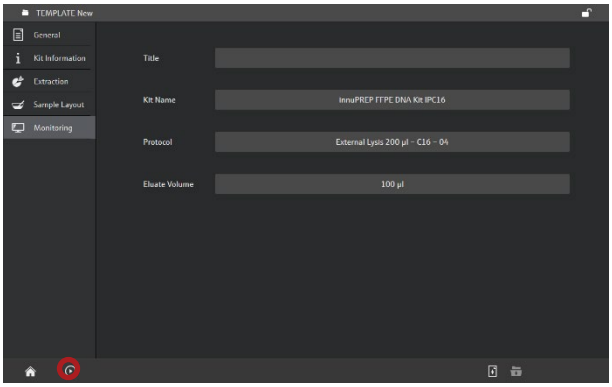
8. Adjust your desired “Eluate Volume“ using the slider or the text field.



NOTE
“Extraction” tab

The recommended elution volume is 50 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
No pellet after first centrifugation step	
Insufficient addition of VCR-1 or VCR-2	Make sure that both VCR-1 and VCR-2 are added to the reaction tube. Make sure that the right volume of VCR-1 and VCR-2 are added.
Insufficient centrifugation	Make sure that centrifugation steps are carried out as describe in the manual. Otherwise repeat centrifugation.
Removing of pellet	Ensure that the pellet is not discarded during removing the supernatant. In some cases the pellet is not seen until the supernatant is removed completely.
Pellet is difficult to dissolve	
Too much addition of VCR-1 or VCR-2	Make sure that both VCR-1 and VCR-2 are added as described in protocol.
Lysis solution not enough added to pellet	Ensure that lysis solution is pipette as described in protocol.
Pipette tip is clogged while dissolving the pellet	Cut the slide edge of pipette tip and try to transfer the pellet as much as possible.
Low concentration of extracted free-circulating DNA	
Too much RNase-free Water	Elute the free-circulating DNA with lower volume of RNase-free water.
No Carrier RNA added	Add Carrier RNA to the sample, as described in the manual above.

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