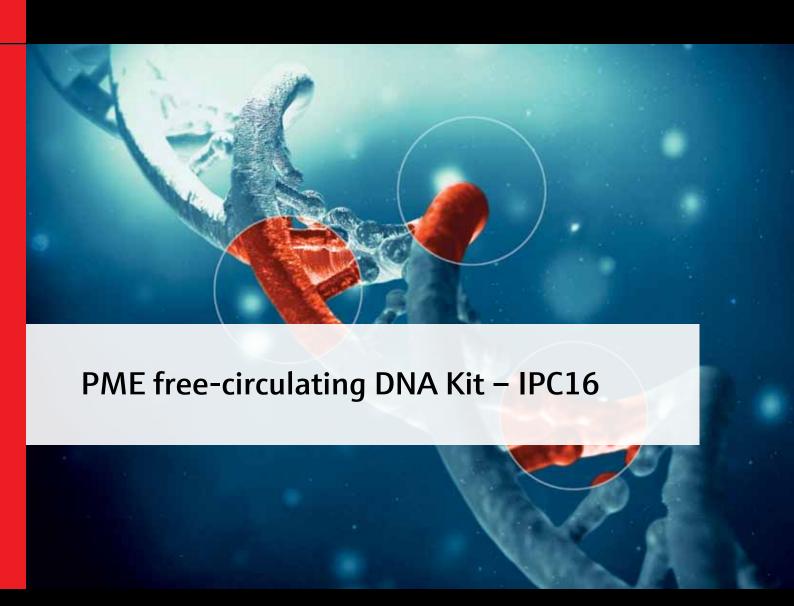
Instructions for UseLife Science Kits & Assays





Order No.:

845-IPS-6116016 16 reactions 845-IPS-6116096 96 reactions 845-IPP-6116016 16 reactions 845-IPP-6116096 96 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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1 Introduction

1.1 Intended use

Free-circulating DNA in serum, plasma or in urine is very interesting 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 nt. Because of these facts, the extraction of cell-free-circulating DNA is difficult. Commercially available 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 is based on new technology, called: PME - Polymer Mediated Enrichment. The procedure doesn't start with sample lysis, like common used methods or kits. The first step is capturing of 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 by 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 $^{\text{\tiny B}}$ 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 low RNase-free water and is now ready to use. The extraction chemistry in combination with the InnuPure® C16 / C16 touch protocol are optimized to get maximum of yield and quality. The whole automatic procedure for the isolation of free-circulating DNA from 1 ml of sample volume needs 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 extreme 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 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 commercial used detection assays.

Please note that in case of using the Carrier RNA the eluates contain free-circulating DNA and Carrier RNA. In 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 not to use the Carrier RNA. Furthermore, Carrier RNA may inhibit PCR reactions. Thus the amount of add 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 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	REF Catalogue number.		
Σ Content Contains sufficient reagents for <n> tests.</n>			
15°C → 30°C	Storage conditions Store at room temperature, unless otherwise specified.		
Ţ <u>i</u>	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. 5).
- 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** and Enrichment Reagent **VCR-1** and 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 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 $^{\circ}$ C to -18 $^{\circ}$ 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 should be stored dry at room temperature (15 $^{\circ}$ C to 30 $^{\circ}$ 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. 11.

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 PME free-circulating 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. 3) (→ "Product specifications" p. 19). 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 Enrichment Reagent VCR-1 at 4 °C to 8 °C!

Store lyophilized Proteinase K at 4 °C to 8 °C!

Divide dissolved **Proteinase K** into aliquots and storage $-22 \,^{\circ}\text{C}$ to $-18 \,^{\circ}\text{C}$ is recommended. Repeated freezing and thawing will reduce the activity dramatically!

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.



STORAGE CONDITIONS

All other components are stored at room temperature.

	Σ 16	∑∑ 96
REF	845-IP[S/P]-6116016	845-IP[S/P]-6116096
Enrichment Reagent VCR-1	2 x 1.2 ml	8 x 1.2 ml
Enrichment Reagent VCR-2	10 ml	2 x 32 ml
Lysis Solution SE	15 ml	60 ml
Carrier RNA	For 1 ml working solution	For 1 ml working solution
RNase-free Water	1 x 2.0 ml	1 x 2.0 ml
Proteinase K	For 2 × 0.3 ml working solution	For 2 × 1.5 ml working solution
Reagent Strip F* (* Depending of order)	16 (pre-filled, sealed)	96 (pre-filled, sealed)

PME free-circulating DNA Kit – IPC16 $\,$ Issue 07 / 2018

Kit components

Reagent Plate F* (* Depending of order)	2 (pre-filled, sealed)	12 (pre-filled, sealed)
Filter Tips	2 × 16	2 × 96
Elution Tubes (0.65 ml)	16	2 × 48
Elution Caps (Stripes)	2	12

Elution Strips	2	12
Manual	1	1
Initial steps	Proteinase K Dissolve Proteinase K by addition of 0.3 ml of ddH ₂ O, mix thoroughly and store as described above.	Proteinase K Dissolve Proteinase K by addition of 1.5 ml of ddH ₂ O, mix thoroughly and store as described above.
	Carrier RNA Dissolve Carrier RNA by addition of 1 ml RNase-free Water; mix thoroughly by pipetting up and down and store as described above!	Carrier RNA Dissolve Carrier RNA by addition of 1 ml RNase- free Water; mix thorough- ly by pipetting up and down and store as de- scribed above!

COMPONENTS NOT INCLUDED IN THE KIT

- ddH₂O for dissolving **Proteinase** K
- 1.5 ml reaction tubes
- 15 ml reaction tubes

7 Recommended steps before starting

- Ensure that the **Proteinase K** and **Carrier RNA have** been prepared according to the instruction (→ "Kit components", p. 11).
- 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.
- Avoid freezing and thawing of starting material.

8 GHS Classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
Reagent Plate/Strip F	Propan-2-ol 50-100 % Polyethylene glycol octylphenol ether 25-50 % Guanidinium thiocyanate 25-50 % Ethanol 50-100 %	Danger	225, 302, 314, 336, 411	101, 102, 103, 210, 303+361+ 353, 305+351+ 338, 310, 405, 501	032
Proteinase K	Proteinase, engyodontium album	Danger	315, 317, 319, 334, 335	101, 102, 103, 261, 280, 305+351+ 338, 342+311, 405, 501	
Lysis Solution SE	Guanidinium chloride 10-25 % Edetic acid 2.5-<10 % Polyethylene glycol octylphenol ether 2.5-<10 %	Danger	315, 318 412	101, 102, 103, 280, 305+351+ 338, 362, 302+352, 403, 501	

GHS Classification

Enrichment Reagent VCR-2	Calcium chloride dihydrate 10-<25 %	! Warning	319	101, 102, 103, 280, 305+ 351+338	
				351+338,	

8.1 Hazard phrases

411

412

225	Highly flammable liquid and vapor.
302	Harmful if swallowed.
314	Causes severe skin burns and eye damage.
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.

Toxic to aquatic life with long lasting effects.

Harmful 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.
362	Take off contaminated clothing.
403	Store in a well-ventilated place.
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+ 353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
305+351+ 338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

9 Product specifications

1. Starting material:

- Serum, plasma, cell culture supernatants or mediums and other cellfree body fluids from 1 ml up to 5 ml
- Urine from 5 ml to 10 ml

2. Time for isolation:

■ Enrichment procedure

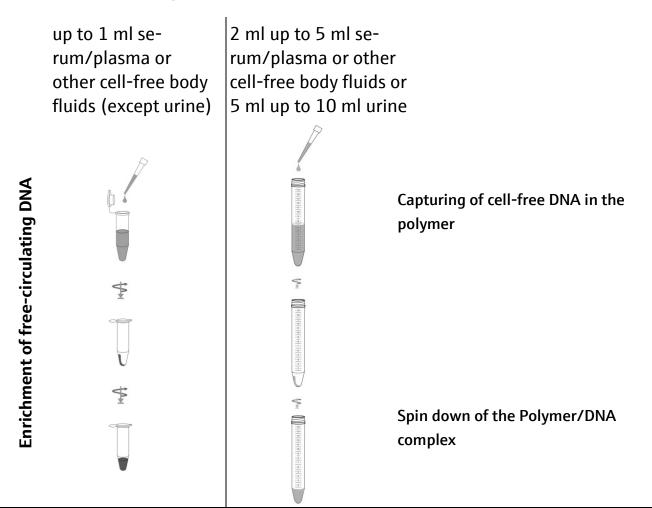
1 ml protocol approx: 10 minutes

5 ml protocol approx: 30 minutes

Automated extraction

Extraction protocol	Protocol on In- nuPure®C16 / C16 touch	Time In- nuPure®C16 / C16 touch	Elution volumes
PME_1ml_C16_04/ PME 1 ml = 05	200 μΙ	70 / 69 min	20-500 μl
PME_5ml_C16_04/ PME 5 ml - 05	200 μΙ	79 / 77 min	20-500 μl

10 General procedure of enrichment and isolation of freecirculating DNA



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

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 μ I of Enrichment Reagent VCR-1 and the sample into a 1.5 ml reaction tube and vortex shortly. Add 150 μ I 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 ddH₂O 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** μ**I** 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!

 Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction ", p. 23). 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. $16,000 \times g$) and work subsequently only with the supernatant.

- 1. Add 100 μ I of Enrichment Reagent VCR-1 and the sample into a 15 ml reaction tube and vortex shortly. Add 600 μ I 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 $4,500 \times g$ ($\sim 5,400 \text{ rpm}$) for 10 minutes, open the tube and remove the supernatant carefully as much as possible.
- 3. Add 5 ml ddH₂O 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 $600 \mu l$ 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 μ l 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 (→ "Preparing Reagent Plate / Strip for automated extraction ", p. 23).

12 Preparing Reagent Plate / Strip for automated extraction

12.1 General filling scheme of reagent reservoir



Cavity 1:	Magnetic particles	Cavity 7:	Washing Solution
Cavity 2:	Empty	Cavity 8:	Washing Solution
Cavity 3:	Empty	Cavity 9:	Washing Solution
Cavity 4:	Empty	Cavity 10:	Washing Solution
Cavity 5:	Empty	Cavity 11:	Empty
Cavity 6:	Binding Solution	Cavity 12:	Elution Buffer

12.2 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.

12.3 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).

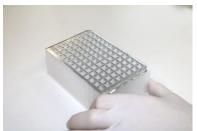
Keep the Reagent Plates or Strips in a horizontal position to avoid spilling of the reagents while piercing of the foil.

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 <u>all</u> cavities of one row per sample!

12.4 Loading the sample to InnuPure® C16 / C16 touch

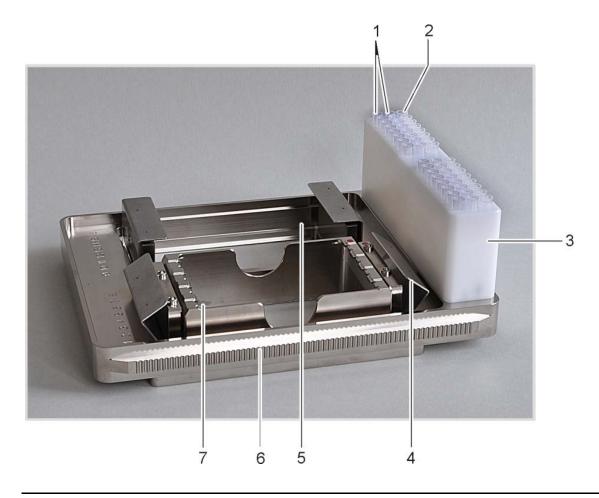
- 1. Ensure the foils of Reagent Plate or Reagent strips have been pierced (→"Preparing Reagent Plate / Strip for automated extraction" p. 23).
- Transfer the whole lysed sample and 25 μl Proteinase K into the third cavity of Reagent Strip or Reagent Plate. Avoid carry-over of solid material!

NOTE

The sample will be processed using the InnuPure® C16 / C16 touch. Please follow the instructions of chapter 13 p. 27.

13 Automated extraction using InnuPure® C16 / C16 touch

13.1 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

13.2 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 μ l use Elution Strips (0.2 ml). For high elution volumes up to 500 μ l use Elution Tubes (0.65 ml) with corresponding Elution Caps (Stripes).

13.3 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 μ l 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

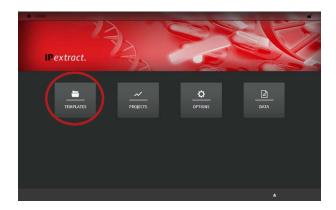
Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 $^{\circ}$ C to -18 $^{\circ}$ C!

13.4 Starting the InnuPure® C16 touch

NOTE

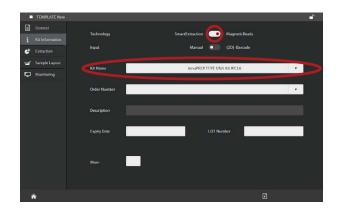
The following instructions describe the necessary steps for the start of the InnuPure® C16 touch. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure® C16 touch.

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] \rightarrow [New Template] \rightarrow [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"!

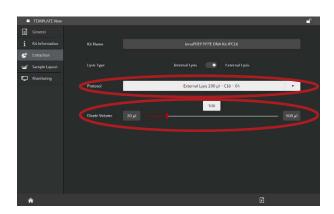


NOTE
"Kit Information" tab

- 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 $50 \mu l$.

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 $-22 \,^{\circ}\text{C}$ to $-18 \,^{\circ}\text{C}$!

14 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 been 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 pel- let	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.				

15 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/μΙ)	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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