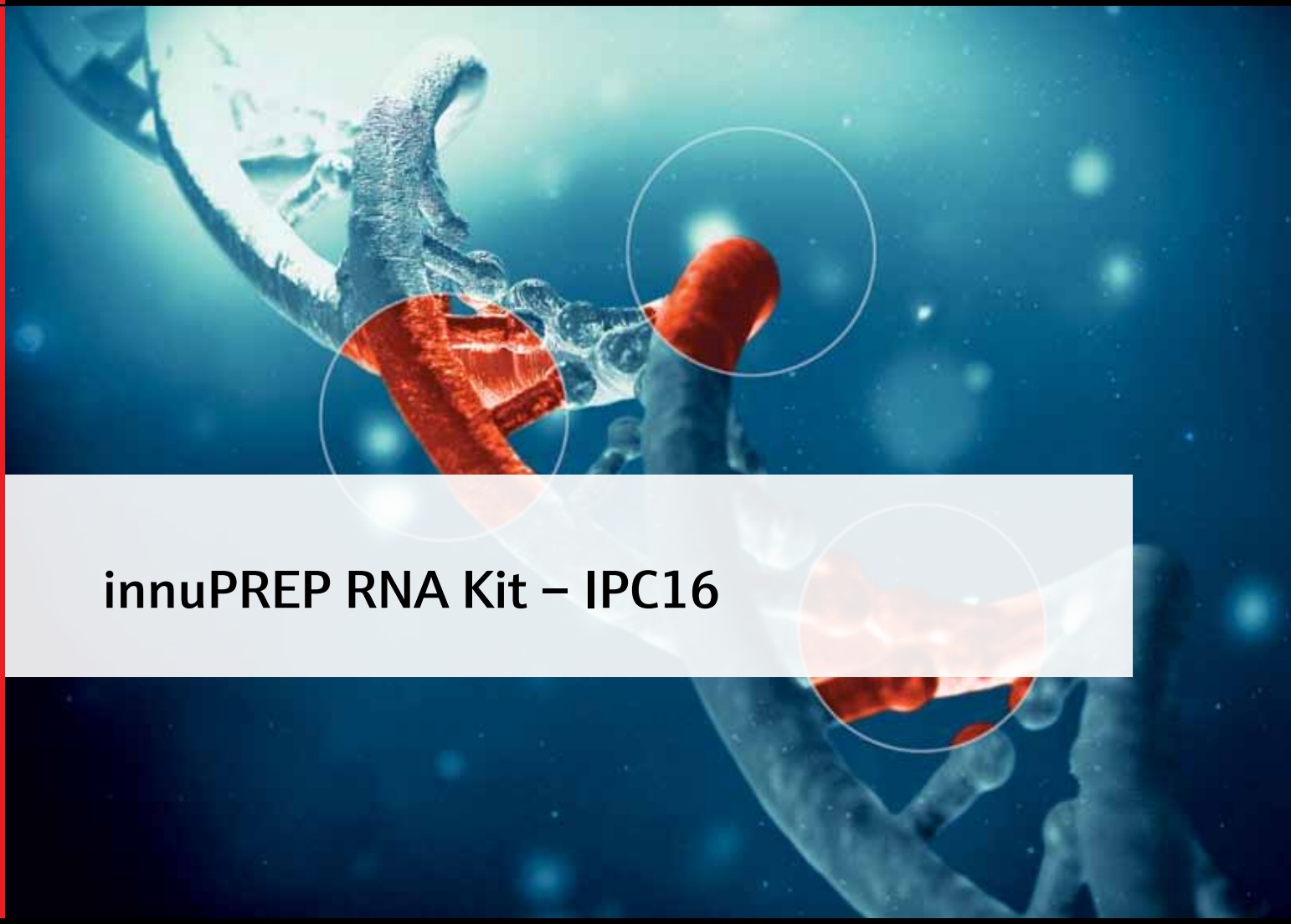
Instructions for Use
Life Science Kits & Assays



innuPREP RNA Kit – IPC16

analytikjena
An Endress+Hauser Company

Order No.:

845-IPP-4116016 16 reactions
845-IPP-4116096 96 reactions
845-IPP-4116480 480 reactions
845-IPS-4116016 16 reactions
845-IPS-4116096 96 reactions

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Contents

1 Introduction

1.1 Intended use

The innuPREP RNA Kit - IPC16 has been designed for automated isolation of total RNA from eukaryotic cells and tissue samples using the InnuPure® C16 / C16 *touch*.

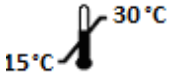
The procedure starts with an external lysis step and subsequent removal of genomic DNA. After the external lysis and incubation step the MAG Suspension F and the samples are transferred into the Reagent Strips or Reagent Plate of the kit, which is already prefilled with all extraction reagents needed for the automated isolation process using the InnuPure® C16 / C16 *touch*. The extraction process is based on binding of the RNA on 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 for downstream applications. The extraction chemistry in combination with the InnuPure® C16 / C16 *touch* protocol are optimized to get a maximum of 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.

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 is designed to be handled by educated personnel in a laboratory environment!

If bottles or plates are damaged or leaking, wear gloves and protective goggles when discarding the bottles or plates in order to avoid any injuries. This kit is to be used with potential infectious samples. 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 General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four hours or more before use. Autoclaving will not inactivate RNase activity completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C followed by autoclaving or heating to 100 °C for 15 minutes to remove residual DEPC.

- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

4 Storage conditions

All components of the innuPREP RNA 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. 10.

5 Functional 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 RNA 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.

6 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. 15). 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.

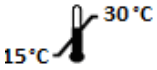
All products sold by the Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE

For research use only!

7 Kit components

IMPORTANT
Store the **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]-4116016	845-IP[S/P]-4116096	845-IPP-4116480
MAG Suspension F	0.25 ml	1.1 ml	5 x 1.1 ml
Lysis Solution RP	10 ml	50 ml	240 ml
Spin Filter	16	2 x 50	10 x 50
Receiver Tubes	16	2 x 50	10 x 50
Reagent Strip K* (* Depending on order)	16 (pre-filled, sealed)	96 (pre-filled, sealed)	--
Reagent Plate K* (* Depending on order)	2 (pre-filled, sealed)	12 (pre-filled, sealed)	12 (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

COMPONENTS NOT INCLUDED IN THE KIT

- 1.5 ml tubes
- 2.0 ml tubes, optional

8 Recommended steps before starting

- Avoid freezing and thawing of starting material.
- Invert the Reagent Plate for 3–4 times and thump it onto a table to collect the prefilled solutions at the bottom of the wells.

9 GHS Classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
Lysis Solution RP	Guanidinium thiocyanate 25–50 % Polyethylene glycol octylphenol ether 10–<25 %	 Danger	302, 314, 412	101, 102, 103, 260,303+361+353, 305+351+338, 310, 405, 501	032
Reagent Plate K	Guanidinium thiocyanate 25–50 % Propan-2-ol 50–100 %	 Danger	225, 314, 336	101, 102, 103, 210, 303+361+353, 305+351+338, 310, 405, 501	032

9.1 Hazard phrases

225 Highly flammable liquid and vapor.

302 Harmful if swallowed.

314 Causes severe skin burns and eye damage.

336 May cause drowsiness or dizziness.

412 Harmful to aquatic life with long lasting effects.

9.2 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.
260	Do not breathe dust/fume/gas/mist/vapors/spray.
310	Immediately call a POISON CENTER/doctor.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
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.

9.3 EU hazard statements

032	Contact with acids liberates very toxic gas.

10 Product specifications

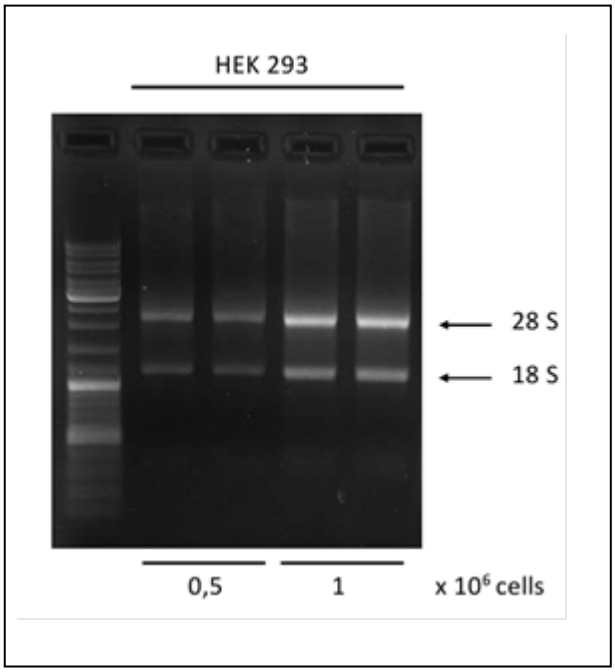
1. Starting material:
   - Eukaryotic cells (max. 5 x 10^6)
   - Tissue samples (max. 20 mg)
   - Tissue samples with a high RNA content (e.g. spleen samples, pancreatic samples, lymph nodes, max. 5 mg)

2. Time for isolation:
   - Lysis: Tissue samples: Depending on type of tissue and homogenization principle
     Eukaryotic cells: approx. 10–20 minutes
   - InnuPure® C16 protocol: approx. 54 minutes

Extraction protocol	Protocol on InnuPure®C16 / C16 touch	Time InnuPure®C16 / C16 touch	Elution volumes
RNA_200_C16_04/ RNA 200 µl – 05	200 µl	55 / 58 min	20–500 µl

3. Typical yield:
   - Depending on amount and condition of the starting material:
     Eukaryotic cells (e.g. NIH 3T3 or HEK 293) up to 20 µg
     Tissue samples (e.g. mouse spleen) up to 50 µg

Example: Preparation of total RNA from different amounts of HEK 293 cells and subsequent electrophoretic separation in a denaturing 1.2 % agarose gel.



11 Protocols

11.1 Isolation of total RNA from eukaryotic cells

NOTE
Do not use more than 5 x 10^6 eukaryotic cells. Higher amounts of eukaryotic cells may clog the membrane of the Spin Filter resulting in a lower yield of total RNA.

1. Pelletize the eukaryotic cells by centrifugation and remove the supernatant as much as possible.
2. Add **450 µl** of **Lysis Solution RP** to the cell pellet and incubate for 2 minutes at room temperature.
3. Resuspend the cell pellet completely by pipetting up and down. Incubate the sample for further 3 minutes at room temperature.

NOTE
To maximize the final yield of total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step. If necessary shake the sample for further 10 minutes at room temperature.

4. Place a Spin Filter D into a Receiver Tube. Transfer the lysed sample onto the Spin Filter D.
5. Centrifuge at 10,000 x g (11,000 rpm) for 2 minutes. Discard the Spin Filter D.

   Do not discard the filtrate, because the filtrate contains the RNA!

IMPORTANT
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Proceed with setting up the Reagent reservoir in section 12.

IMPORTANT
The lysed sample will be processed using the InnuPure® C16 / C16 *touch*. Please follow the instruction of the manual from point 12 on page 22!

11.2 Isolation of total RNA from tissue samples

IMPORTANT
Please note that up to 20 mg of tissue material can be processed.
To maximize the final yield of total RNA a complete homogenization of tissue sample is important!
Avoid freezing and thawing of tissue samples!

1. Homogenization of starting material

IMPORTANT
To maximize the final yield of total RNA a complete homogenization of tissue sample is important!

For the homogenization of tissue sample it is possible to use commercially available rotor-stator homogenizer or bead mills. It is also possible to disrupt the starting material using mortar and pestle in liquid nitrogen and grind the tissue sample to a fine powder.

A. Homogenization of the tissue sample using a rotor-stator homogenizer

1. Transfer the weighed amount of fresh or frozen starting material in a suitable reaction vessel for the homogenizer.
2. Add **450 µl Lysis Solution RP**.
3. Homogenize the sample.
4. Transfer the homogenized tissue sample into a 1.5 ml reaction tube and place the sample in Lysis Solution RP for longer storage at -22 °C to -18 °C or use the sample immediately for isolation of total RNA following the protocol step 2.

B. Disruption of the tissue sample using a mortar and pestle and liquid nitrogen

1. Transfer the weighed amount of fresh or frozen starting material under liquid nitrogen and grind the material to a fine tissue powder.
2. Transfer the powder into a 1.5 ml reaction tube. Don't allow the sample to thaw!
3. Add **450 µl Lysis Solution RP** and incubate the sample for appropriate time for a further lysis under continuous shaking.
4. Finally place the sample under Lysis Solution RP for longer storage at -22 °C to -18 °C or use the sample immediately for isolation of total RNA following protocol step 2.

2. After homogenization please check, that the starting material is completely disrupted.
3. Spin down unlysed material by centrifugation at maximum speed for 1 minute.
4. Place a Spin Filter D into a Receiver Tube and transfer the supernatant of the lysed sample onto the Spin Filter D.
5. Centrifuge the Receiver Tube at 10,000 x g (11,000 rpm) for 2 minutes. Discard the Spin Filter D.

Do not discard the filtrate, because the filtrate contains the RNA!

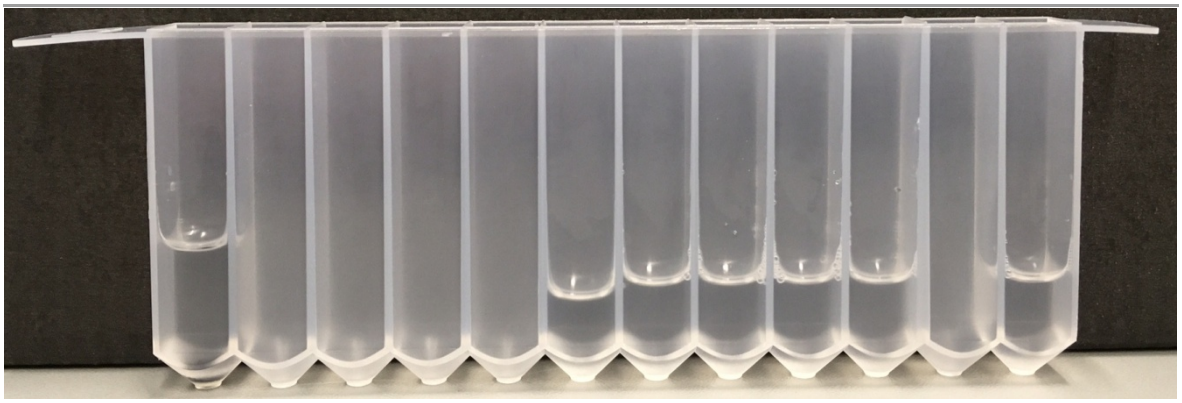
IMPORTANT
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Proceed with setting up the Reagent reservoir in section 12.

IMPORTANT
The lysed sample will be processed using the InnuPure® C16 / C16 *touch*. Please follow the instruction of the manual from point 12 on page 22!

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 Strips

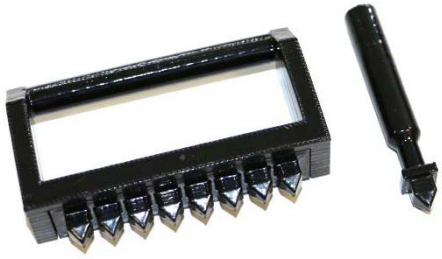
NOTE
According to transport regulations Reagent Reservoirs are wrapped into plastic bags only when transported by airplane.

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

Carefully open the overpack of Reagent Plates 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!

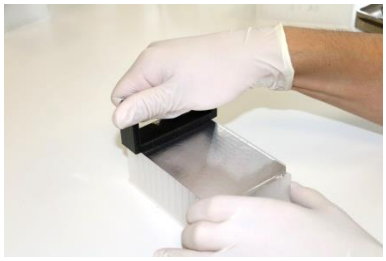
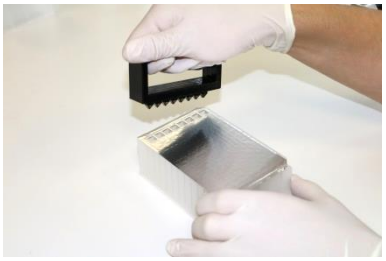
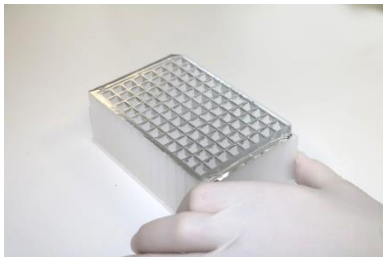


Reagent Plates or Strips are prefilled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (single piercer or 8fold piercer).

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

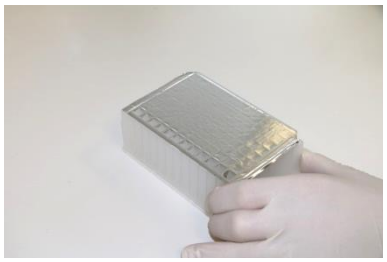
Open all cavities (one row per sample).

Using 8 samples in parallel

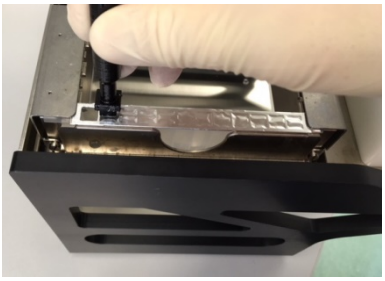
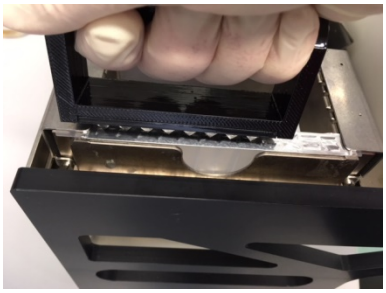
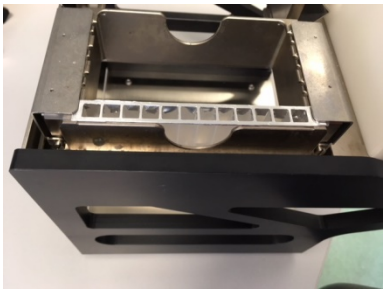




Using single samples





Using Reagent Strips

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

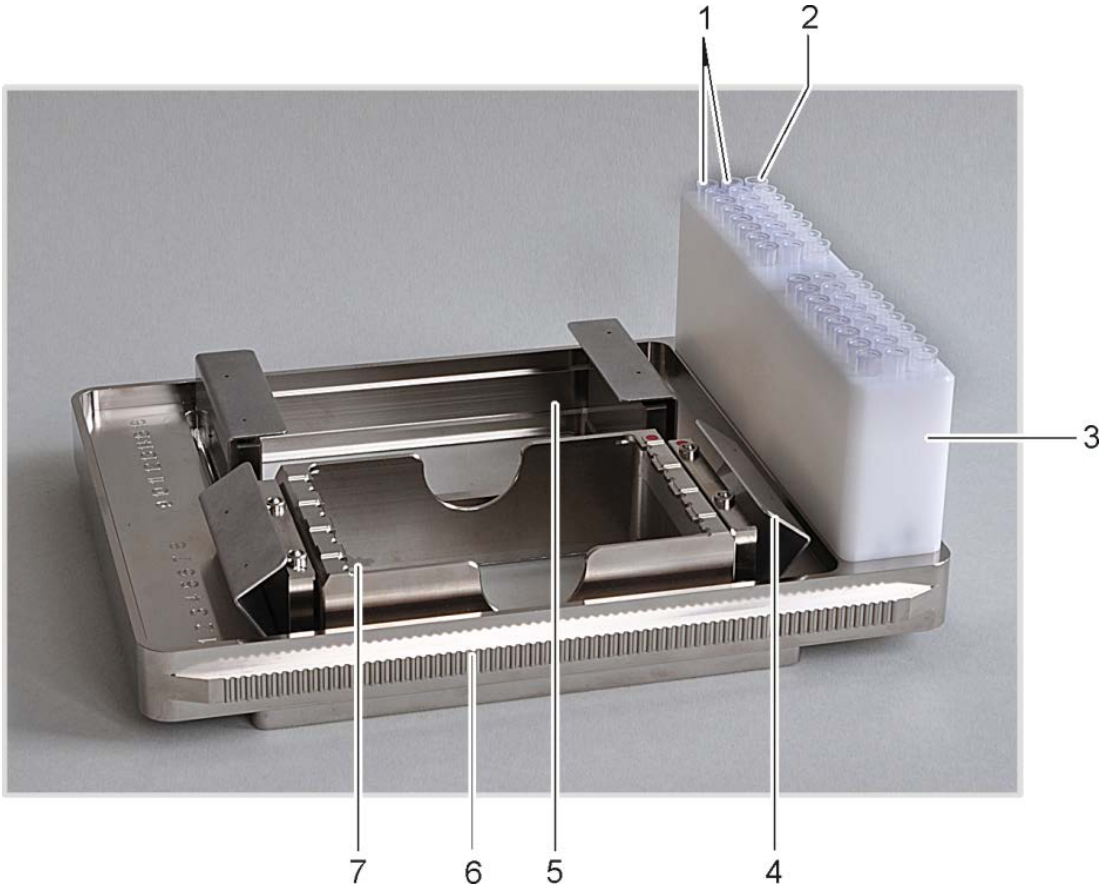
12.4 Loading the sample to InnuPure® C16 / C16 touch

1. Ensure the foils of Reagent Plate or Reagent strips have been pierced (→ „Preparing Reagent Plate / Strip for automated extraction“ p. 22).
2. Transfer **10 µl** of **MAG Suspension F** directly into the liquid of the **first cavity** of Reagent Strip or Reagent Plate.
3. Transfer **400 µl** of the **lysed sample** 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 touch

13.1 Sample tray of InnuPure® C16 touch



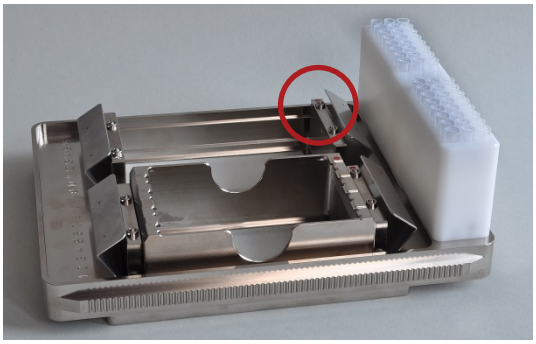
No. 1:	Filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Holding-down clamp
No. 5:	Sample block for Reagent Plates
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for Reagent Strips (optional)

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 *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.



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

3. Fold down the holding-down clamp to prevent the Reagent Plates 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).

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
Standard	RNA_200_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.

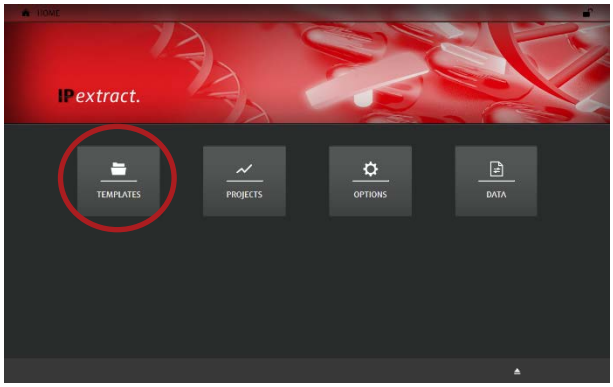
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

13.4 Starting the InnuPure® C16 touch

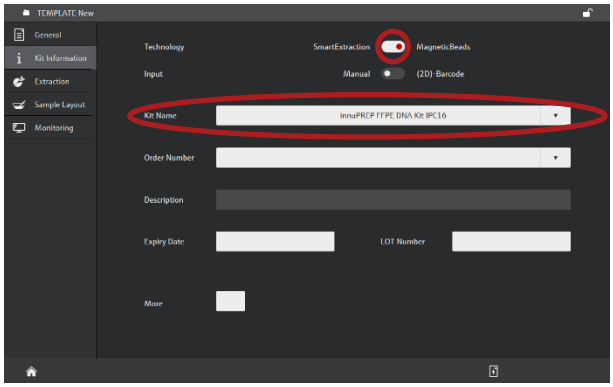
NOTE
The following instructions describe the necessary steps for the start of the InnuPure® C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure® C16 *touch*.

9. 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*

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

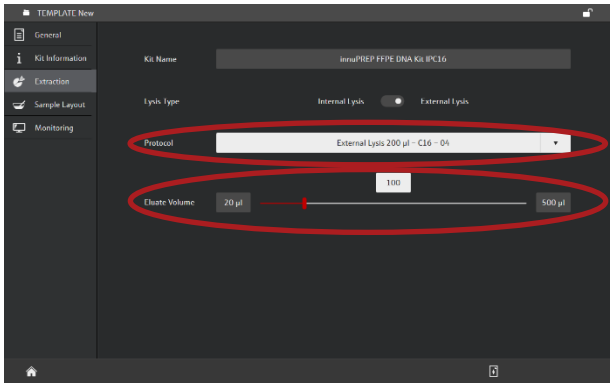


NOTE
“Kit Information” tab

14. Enter optional information in the tab “Kit Information”
15. Choose the tab “Extraction” and choose the desired “Protocol”

Extraction procedure	Protocol on InnuPure® C16 touch
Standard	RNA 200 µl – 05

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



NOTE
“Extraction” tab

The recommended elution volume is 100 µl.

14 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted RNA	
Content of viral nucleic acid in sample insufficient.	Use the right volume of starting material 400 µl. Mix **MAG Suspension F** well before usage!
Insufficient lysis of starting material.	Ensure to use the required volume of Proteinase K for current protocol.
Inadequate extraction.	Inhibiting substances in starting material. Please use the kit only for samples that match the requirements declared in “Product specifications”.
Poor quality of extracted RNA	
Extracted RNA contains genomic DNA	Content of DNA in starting material too high. Reduce amount of starting material and/or perform DNAse digestion.

15 Related Products

Name	Amount	Order No.
Products for nucleic acid purification		
innuPREP DNA Kit – IPC16	16 rxn (Plates)	845-IPP-2016016
	96 rxn(Plates)	845-IPP-2016096
	480 rxn(Plates)	845-IPP-2016480
Products for PCR & Gel Electrophoresis		
innuPREP DOUBLEpure Kit	10 rxn	845-KS-5050010
	50 rxn	845-KS-5050050
	250 rxn	845-KS-5050250
innuPREP Gel Extraction Kit	10 rxn	845-KS-5030010
	50 rxn	845-KS-5030050
	250 rxn	845-KS-5030250
innuPREP PCRpure Kit	10 rxn	845-KS-5010010
	50 rxn	845-KS-5010050
	250 rxn	845-KS-5010250
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
50x inNucleotide Mix (1.5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
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
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3x 1.0 ml	845-ST-3010003
	6x 1.0 ml	845-ST-3010006

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