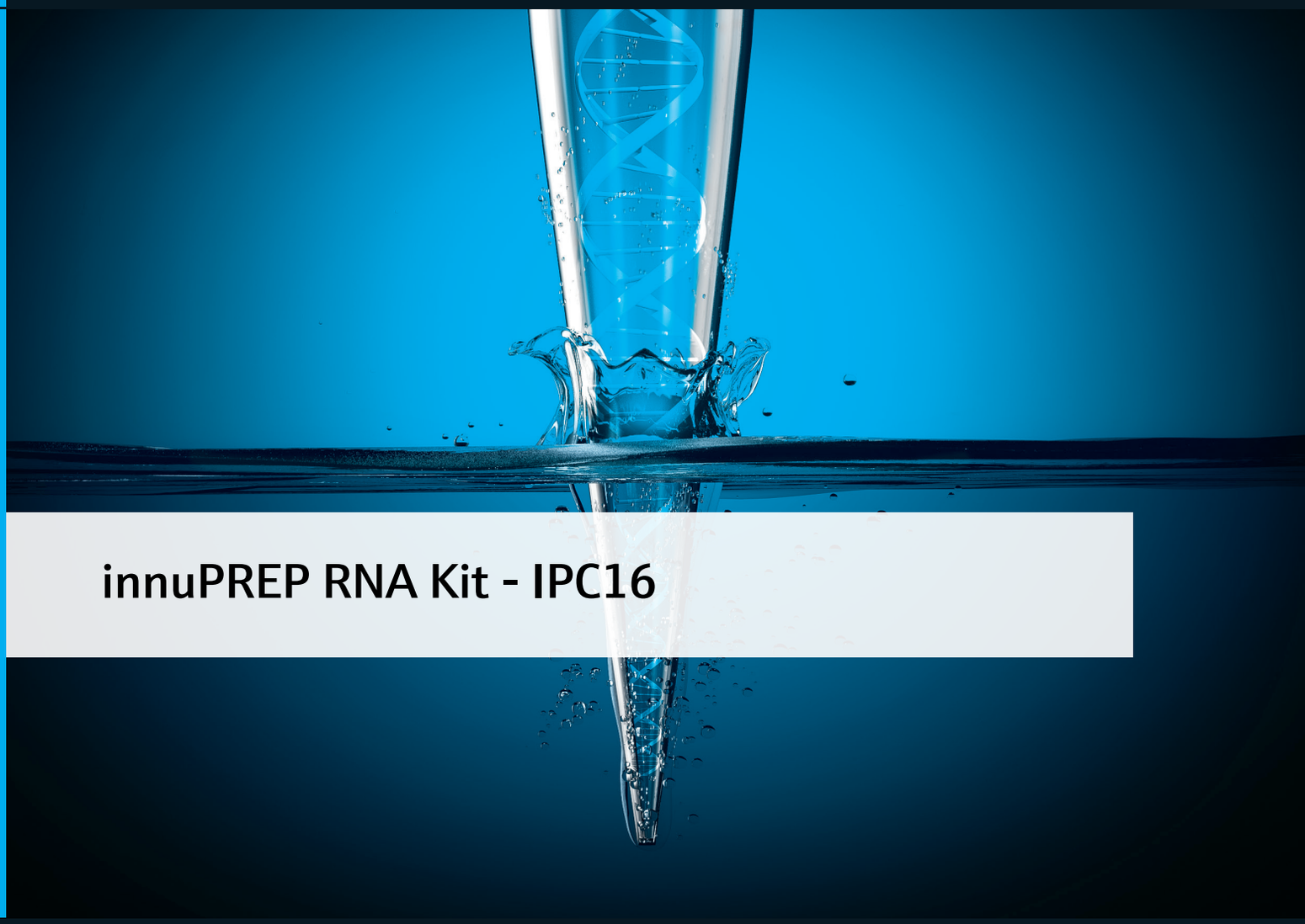
Instructions for Use
Life Science Kits & Assays



innuPREP RNA Kit - IPC16

Innuscreen GmbH

Order No.:

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

Publication No.: HB_IP-4116_e_220411

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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 and the kit

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

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> tests.
15 °C 30 °C	**Storage conditions** Store at room temperature, unless otherwise specified.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → “Notes on the use of this manual and the kit” p. 3).
- 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 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 on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

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

The kit is shipped at ambient temperature.

Upon arrival store **MAG Suspension F** at 4 °C to 8 °C.

All other 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.

5 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP RNA Kit - IPC16 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.

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 (→ “Product specifications” p. 9). 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.

NOTE

The kit is for research use only!

7 Kit components

7.1 Included kit components

	Σ 16	Σ 96	Σ 480
REF	845-IPS-4116016[a] 845-IPP-4116016[b]	845-IPS-4116096[a] 845-IPP-4116096[b]	845-IPP-4116480[b]
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[a]	16 (pre-filled, sealed)	96 (pre-filled, sealed)	--
Reagent Plate K[b]	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

7.2 Components not included in the kit

- 1.5 ml tubes
- 2.0 ml tubes, optional

8 Initial steps before starting

- 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.
- 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 Product specifications

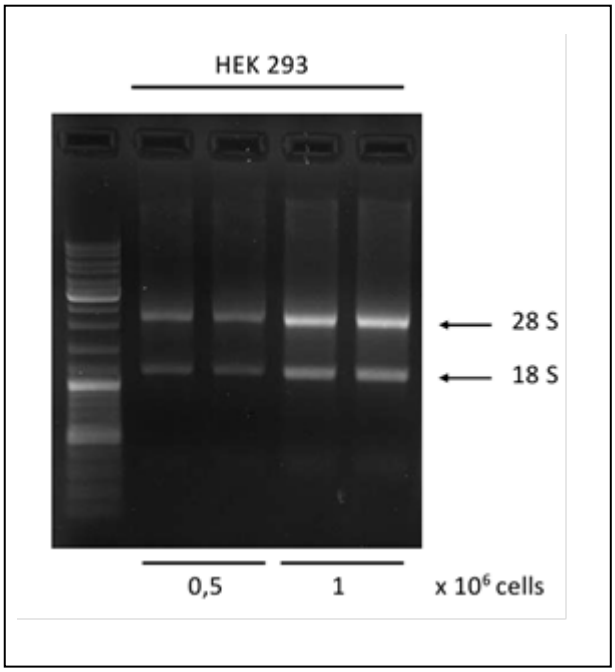
1. **Starting material**
   - Eukaryotic cells (max. 5 x 10^6)
   - Tissue samples (max. 20 mg)
   - Tissue samples with a high RNA content (e.g. spleen samples, pancreatic samples, lymph nodes, max. 5 mg)
2. **Time for isolation**
   - Lysis: Tissue samples: Depending on type of tissue and homogenization principle

     Eukaryotic cells: approx. 10–20 minutes
   - InnuPure C16 protocol: approx. 54 minutes

Extraction protocol	Protocol on InnuPureC16 / C16 touch	Time InnuPureC16 / 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 RNA
   - Tissue samples (e.g. mouse spleen) up to 50 µg RNA

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



10 Protocols for isolation of total RNA

10.1 Isolation 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 14!

10.2 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: 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.

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

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

2. Transfer the weighed amount of fresh or frozen starting material in a suitable reaction vessel for the homogenizer.
3. Add **450 µl Lysis Solution RP**.
4. Homogenize the sample.
5. 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

2. Transfer the weighed amount of fresh or frozen starting material under liquid nitrogen and grind the material to a fine tissue powder.
3. Transfer the powder into a 1.5 ml reaction tube. Don't allow the sample to thaw!
4. Add **450 µl Lysis Solution RP** and incubate the sample for appropriate time for a further lysis under continuous shaking.
5. 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.

6. After homogenization please check, that the starting material is completely disrupted.
7. Spin down unlysed material by centrifugation at maximum speed for 1 minute.
8. Place a Spin Filter D into a Receiver Tube and transfer the supernatant of the lysed sample onto the Spin Filter D.
9. 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!

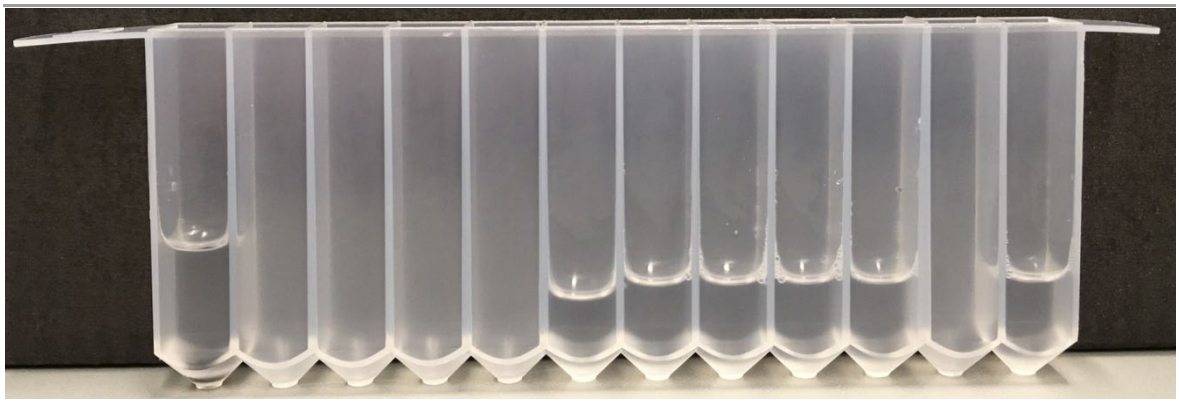
IMPORTANT
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

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 14!

11 Preparing Reagent Plate / Strip for automated extraction

11.1 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

11.2 Unpacking of Reagent Plate or Reagent Strips

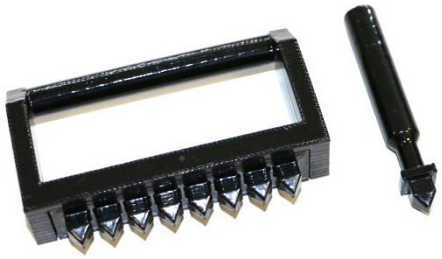
NOTE
According to transport regulations Reagent Reservoirs are wrapped into plastic bags only when transported by airplane.

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

Carefully open the overpack of Reagent Plates by using scissors.

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!

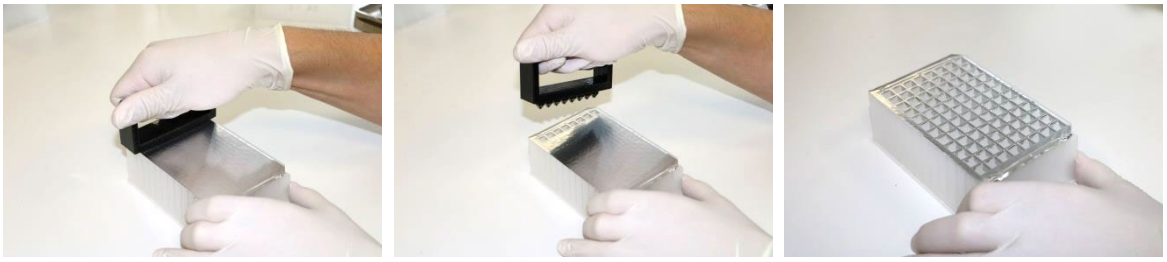


Reagent Plates or Strips are prefilled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (single piercer or 8fold piercer).

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

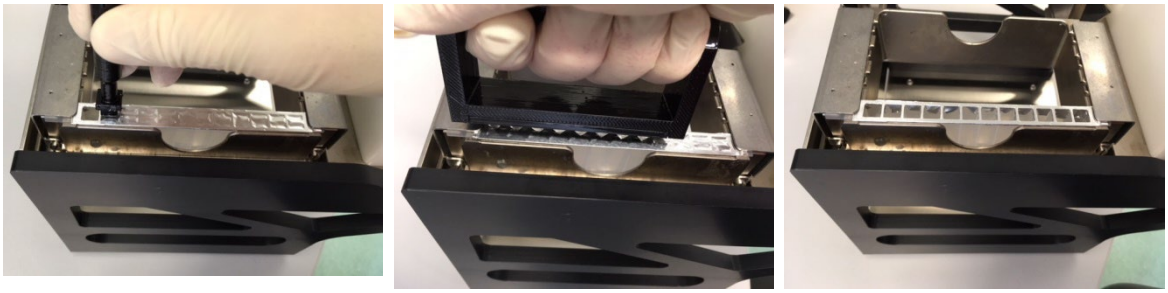
Open all cavities (one row per sample).

Using 8 samples in parallel



Using single samples

Using Reagent Strips



IMPORTANT

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

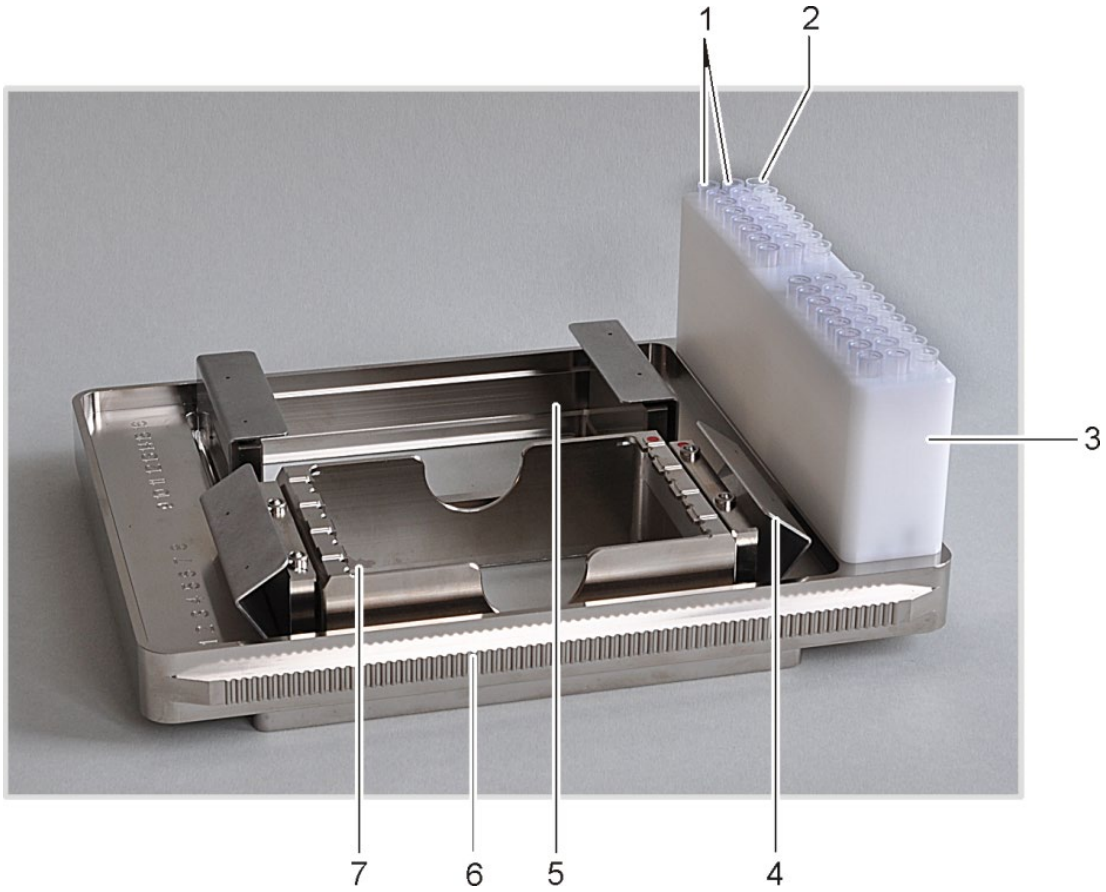
11.4 Loading the sample to InnuPure C16 / C16 touch

1. Ensure the foils of Reagent Plate or Reagent strips have been pierced (→ „Preparing Reagent Plate / Strip for automated extraction“ p. 14).
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. 18.

12 Automated extraction using InnuPure C16 touch

12.1 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)

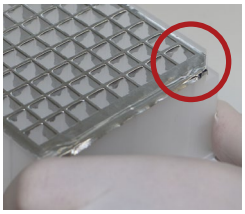
12.2 Preparing sample tray of InnuPure C16 / C16 touch

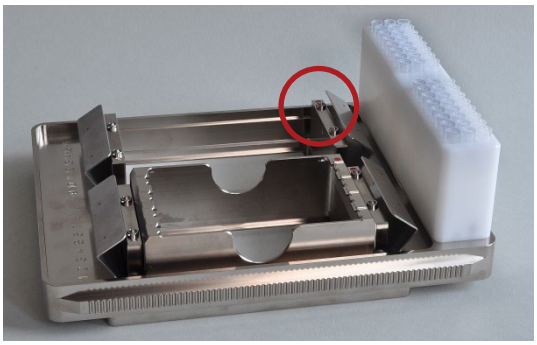
NOTE
The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more strips as number of samples!

1. Place the InnuPure C16 *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.

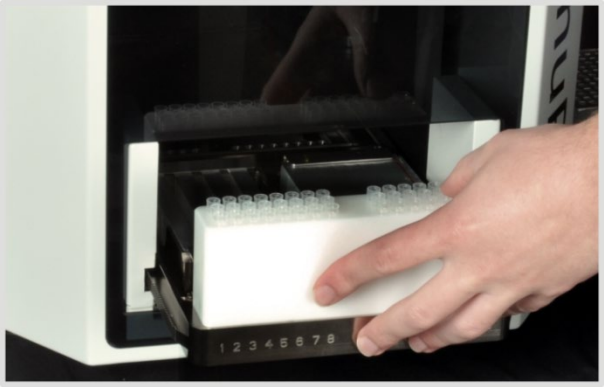
5. Place the Elution Tubes into the wider drill hole at the edge of the tip block. Empty sample positions do not need to be filled.

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).

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	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!

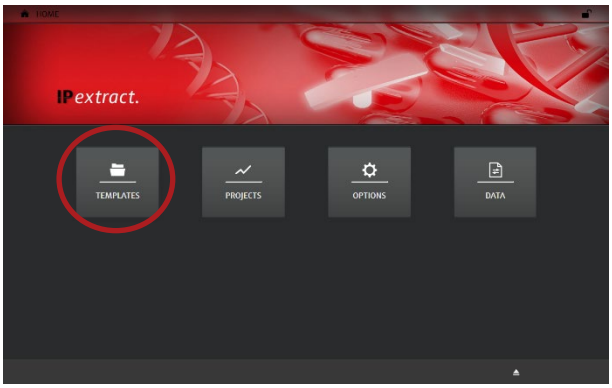
7. Remove the sample tray from the adapter of the InnuPure C16 and place it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

12.4 Starting the InnuPure C16 touch

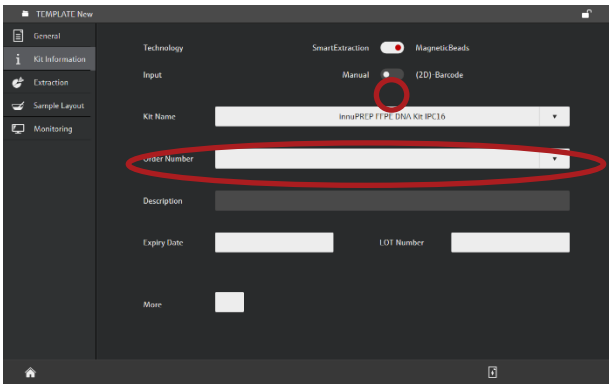
NOTE
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

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”!



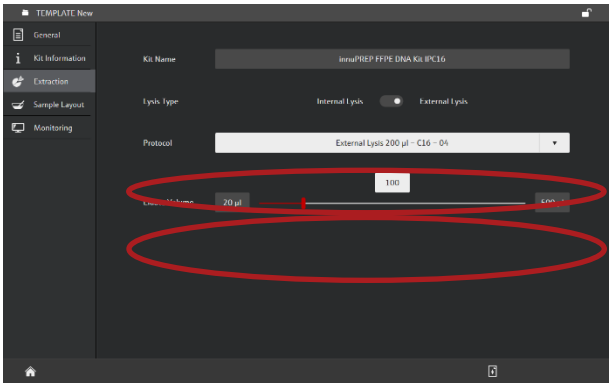
NOTE
“Kit Information” tab

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.

13 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.

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