Instructions for UseLife Science Kits & Assays





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1 Introduction

1.1 Intended use

The innuPREP Virus DNA/RNA Kit – IPC16, non-filled kit has been designed for the fully automated isolation of both viral DNA and RNA from 200 μ l, 400 μ l and 600 μ l of serum, plasma and other cell free samples. Furthermore, 200 μ l of cell culture supernatants, fecal samples, swabs and other relevant starting materials can be used for isolation. The extraction procedure is based on a new patented chemistry. The kit is designed to be handled by educated personnel in a laboratory environment.

For the liquid samples all steps of the extraction process are fully automated and run completely on the InnuPure C16 / C16 touch. The samples are transferred into the Reagent Plates of the kit, which must be prefilled with all reagents needed for the

extraction procedure. The extraction process runs automatically on the InnuPure C16 / C16 touch. The extraction is based on binding of DNA and/or RNA to surface-modified magnetic particles. After several washing steps the nucleic acids are eluted from the magnetic particles with RNase-free water and are ready to be used in downstream applications. The extraction chemistry in combination with the InnuPure C16 / C16 touch protocols is optimized to get maximum yield and quality.

In order to both optimize recovery of minute amounts of nucleic acids within the sample and to verify the successful extraction of nucleic acids the kit contains a Carrier Mix consisting of a carrier RNA as well as internal control DNA (IC DNA) and RNA (IC RNA). The IC DNA and IC RNA can be detected by real-time PCR with a corresponding real-time PCR detection kit.

CONSULT INSTRUCTIONS 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	REF Catalogue number.
\sum_{N}	Content Contains sufficient reagents for <n> tests.</n>
15°C	Storage conditions Store at room temperature, unless otherwise specified.
[]i	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
\Box	Expiry date
LOT	Lot number The number of the kit charge.
	Manufactured by Contact information of manufacturer.
(2)	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. 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.

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!

Do not eat or drink components of the kit!

The kit is designed to 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. This kit is to be used with potentially infectious human samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulations.

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.

- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free water.
- 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 kit are shipped at room temperature.

Upon arrival, store lyophilized and dissolved Proteinase K at 4 °C to 8 °C.

Store lyophilized **Carrier Mix** at -22 °C to -18 °C. Store dissolved Carrier Mix in aliquots at -22 °C to -18 °C. Do not freeze and thaw the Carrier Mix more than 3 times.

Store the MAG Suspension at 4 °C to 8 °C.

The mixture of Lysis Solution V and Carrier Mix is stable for a maximum of 7 days if stored at 4 °C to 8 °C.

All other components of the innuPREP Virus DNA/RNA Kit – IPC16, non-filled 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 are at room temperature. If there are any precipitates within the provided solutions, they can be dissolved 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 this 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 Virus DNA/RNA 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 dial +49 30 9489 3380. For support in 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 material than those referred to in the manual (→ "Product specifications", p. 11). Since the performance characteristics of IST Innuscreen GmbH kits have only been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits when using other protocols than those described below. IST Innuscree 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 Components included in the kit

	\(\sum_{16}\)	∑∑ 96	∑∑ 480
REF	845-PPP-7116016	845-PPP-7116096	845-PPP-7116480
MAG Suspension	1 ml	2 x 1.5 ml	14 ml
Proteinase K	For 1 x 1.5 ml working solution	For 4 x 1.5 ml working solution	For 17 x 1.5 ml working solution
Carrier Mix	For 1 x 1.25 ml working solution	For 1 x 1.25 ml working solution	For 5 x 1.25 ml working solution
Lysis Solution V	15 ml	65 ml	320 ml
Binding Solution V	30 ml	150 ml	750 ml
Washing Solution A	30 ml	120 ml	600 ml
Washing Solution B2 (conc.)	10 ml	50 ml	240 ml
RNase-free Water	30 ml	2 x 80 ml	2 x 400 ml
Deep Well Plate (2.0 ml)	2	12	60
Filter Tips	2 x 16	2 x 96	10 x 96
Elution Tubes (0.65 ml)	16	2 x 48	10 x 48
Elution Caps (Stripes)	2	12	5 x 12
Elution Stripes	2	12	5 x 12
Manual	1	1	1

7.2 Components not included in the kit

- 1.5 ml and 15 ml tubes
- ddH₂O for dissolving **Proteinase** K
- 96-99.8 % ethanol (molecular biology grade, undenaturated)
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄) for Protocol 6
- Physiological saline (0,9 % NaCl) for Protocol 5
- Lysis Tubes P (innuSPEED Lysis Tube P, 845-CS-1020250) for Protocol 8

8 Usage of Carrier Mix

In addition to carrier RNA, the **Carrier Mix** contains an Internal Control DNA and Internal Control RNA (IC DNA and IC RNA). Both can be detected by real-time PCR using the corresponding assay.

Name	Amount	Order No.
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

If customized extraction controls are used, please add these components to the mixture of Lysis Solution V / Carrier Mix (see the specific protocol → "Protocols for isolation of viral DNA and RNA", p. 14).

9 Product specifications

- 1. Starting material
- Cell-free body fluids (serum, plasma, cerebrospinal fluid, liquor);
 200 μl, 400 μl or 600 μl sample volume.
- Cell culture supernatants; max. 200 μl sample volume.
- Swab samples; max. 200 μl sample volume.
- Stool samples; max. 200 μl sample volume.

2. Time for isolation

Extraction protocol	Protocol on IPC16 / C16 touch	Time IPC16 / C16 touch	Elution volumes
Int_Lysis_200_C16_04/ Internal Lysis 200 µl – C16 – 05	200 µl	79 / 77 min	20-500 μl
Int_Lysis_200_Fast_C16_04/ Internal Lysis 200 µl – Fast – C16 – 05	200 µl	59 / 58 min	20-500 μl
Int_Lysis_200_Ultra_Fast_C16_04/ Internal Lysis 200 µl – Ultra Fast – C16 – 05	200 µl	33 / 31 min	20-500 µl
Int_Lysis_400_C16_04/ Internal Lysis 400 µl – C16 – 05	400 μl	90 / 89 min	20-500 μl
Int_Lysis_600_C16_04/ Internal Lysis 600 µl – C16 – 05	600 µl	98 / 97 min	20-500 μl

10 Initial steps before starting

- Add 1.5 ml ddH₂O to each vial of lyophilized **Proteinase K**, mix thoroughly and store as described above.
- Add the indicated amount of absolute ethanol to Washing Solution B2 (conc.) and mix thoroughly. Keep the bottle always firmly closed!

```
845-PPP-8016016 Add 15 ml ethanol to 10 ml Washing Solution B2 (conc.)

845-PPP-8016096 Add 75 ml ethanol to 50 ml Washing Solution B2 (conc.)

845-PPP-8016480 Add 360 ml ethanol to 240 ml Washing Solution B2 (conc.)
```

- Add 1.25 ml RNase-free Water to each vial of Carrier Mix, mix thoroughly and store as described above.
- Avoid freezing and thawing of starting material.
- Prepare mixture of Lysis Solution V and Carrier Mix according to the table in the respective protocol and store as described above.

11 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 925 µl of RNase-free water) and also add MAG Suspension, Sample and Proteinase K as described in the chapter "Protocols for isolation of viral DNA and RNA" (p. 14).

Deep Well Plate	Row No.	Solution	Volume per cavity
	1	RNase-free Water	925 µl
	2	empty	
	3	empty	
	4	empty	
	5	empty	
	6	Binding Solution V	1400 μΙ
	7	Washing Solution A	600 µl
	8	Washing Solution A	600 µl
	9	Washing Solution B2	600 µl
	10	Washing Solution B2	600 µl
1 M O O W W O O	11	empty	
	12	RNase-free Water	600 µl

12 Protocols for isolation of viral DNA and RNA

NOTE

We recommend the addition of Carrier Mix. Ensure the **Carrier Mix** has been prepared as described. The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.

NOTE

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

- 12.1 <u>Protocol 1</u>: Isolation from 200 μl cell-free body fluids (serum, plasma, cerebrospinal fluid, liquor)
 - 1. Transfer **25** μ**I** of **MAG Suspension** directly into the liquid of the <u>first</u> <u>cavity</u> of the Reagent Plate.
 - 2. Prepare the mixture of Lysis Solution V and Carrier Mix according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	4.0 ml	24.0 ml	250 μl x n samples
Carrier Mix	0.2 ml	1.2 ml	12.5 μl x n samples
Final volume	4.2 ml	25.2 ml	262.5 µl x n samples

- 3. Transfer **200** µl Lysis Solution V / Carrier Mix directly into the <u>third</u> <u>cavity</u> of the Reagent Plate.
- 4. Add 200 μl of the sample to the third cavity of the Reagent Plate containing Lysis Solution V / Carrier Mix.
- 5. Add **20 µl Proteinase K** to the **third cavity** of the Reagent Plate.
- 6. The sample will be processed using the InnuPure C16 / C16 touch. Please follow the instructions of chapter 13 p. 21.

12.2 Protocol 2: Isolation from 400 μl cell-free body fluids

- 1. Transfer **25** μ**I** of **MAG Suspension** directly into the liquid of the <u>first</u> cavity of the Reagent Plate.
- 2. Prepare the mixture of Lysis Solution V and Carrier Mix according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	6.9 ml	41.3 ml	430 μl x n samples
Carrier Mix	0.168 ml	1.008 ml	10.5 μl x n samples
Final volume	7.0 ml	42.3 ml	440.5 μl x n samples

- 3. Transfer **400** µl Lysis Solution V / Carrier Mix directly into the <u>third</u> cavity of the Reagent Plate.
- 4. Add **400** μ**I** of the sample to the <u>third cavity</u> of the Reagent Strip or Reagent Plate containing Lysis Solution V / Carrier Mix.
- 5. Add **30** µl **Proteinase** K to the **third cavity** of the Reagent Plate.
- 6. The sample will be processed using the InnuPure C16 / C16 touch. Please follow the instructions of chapter 13 p. 21.

12.3 Protocol 3: Isolation from 600 µl cell-free body fluids

- 1. Transfer 25 μl of MAG Suspension directly into the liquid of the <u>first</u> cavity of the Reagent Plate.
- 2. Prepare the mixture of Lysis Solution V and Carrier Mix according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	10.4 ml	62.4 ml	650 μl x n samples
Carrier Mix	0.2 ml	1.0 ml	10.5 μl x n samples
Final volume	10.6 ml	63.4 ml	660.5 µl x n samples

3. Transfer **600** µl Lysis Solution V / Carrier Mix directly into the <u>third</u> cavity of the Reagent Plate.

- 4. Add **600** μl of the sample to the <u>third cavity</u> of the Reagent Plate containing Lysis Solution V / Carrier Mix.
- 5. Add **50** μ**l** Proteinase K to the third cavity of the Reagent Plate.
- 6. The sample will be processed using the InnuPure C16 / C16 touch. Please follow the instructions of chapter 13 p. 21.

12.4 Protocol 4: Isolation from 200 μl of cell culture supernatants

- 1. Transfer **25** μ**I** of **MAG Suspension** directly into the liquid of the <u>first</u> <u>cavity</u> of the Reagent Plate or Reagent Strip.
- 2. Prepare the mixture of Lysis Solution V and Carrier Mix according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	4.0 ml	24.0 ml	250 µl x n samples
Carrier Mix	0.2 ml	1.2 ml	12.5 μl x n samples
Final volume	4.2 ml	25.2 ml	262.5 µl x n samples

- 3. Transfer **200** µl Lysis Solution V / Carrier Mix directly into the <u>third</u> <u>cavity</u> of the Reagent Plate.
- 4. Add **200** μ**I** of the sample to the <u>third cavity</u> of the Reagent Plate containing Lysis Solution V / Carrier Mix.
- 5. Add **20 µl Proteinase K** to the **third cavity** of the Reagent Plate.
- 6. The sample will be processed using the InnuPure C16 / C16 touch. Please follow the instructions of chapter 13 p. 21.

12.5 <u>Protocol 5</u>: Isolation from 200 μl of swab samples (e.g. Influenza A extraction)

- 1. Place the swabs into 1.5 ml reaction tubes containing 500 μl physiological saline (0.9 % NaCl, not included in the kit), incubate for 10 minutes and shake the swabs vigorously inside the solution. Squeeze the swabs against the wall of the tube before removing them.
- 2. Transfer **25 μl** of **MAG Suspension** directly into the liquid of the <u>first</u> <u>cavity</u> of the Reagent Plate.
- 3. Prepare the mixture of Lysis Solution V and Carrier Mix according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	4.0 ml	24.0 ml	250 μl x n samples
Carrier Mix	0.2 ml	1.2 ml	12.5 μl x n samples
Final volume	4.2 ml	25.2 ml	262.5 μl x n samples

- 4. Transfer 200 μl Lysis Solution V / Carrier Mix directly into the <u>third</u> <u>cavity</u> of the Reagent Plate.
- 5. Transfer **200** μ**I** of the sample to the <u>third cavity</u> of the Reagent Plate containing Lysis Solution V / Carrier Mix.
- 6. Add 20 µl Proteinase K to the third cavity of the Reagent Plate.
- 7. The sample will be processed using the InnuPure C16 / C16 touch. Please follow the instructions of chapter 13 p. 21.

12.6 <u>Protocol 6</u>: Isolation from 200 μl of stool samples (e.g. Norovirus extraction)

NOTE

In some cases, the initial fecal sample is mixed with special buffers for subsequent ELISA detection of different viruses.

In this case use Protocol 7.

- 1. **Transfer 25 μl** of **MAG Suspension** directly into the liquid of the <u>first</u> <u>cavity</u> of the Reagent Plate.
- 2. Transfer about 50-100 mg of the stool sample into a 1.5 ml reaction tube and add 250 μ l PBS (not included in the kit).
- 3. Vortex the sample for 5 seconds and centrifuge it at max. speed for 3 minutes.
- 4. Prepare the mixture of Lysis Solution V and Carrier Mix according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	4.0 ml	24.0 ml	250 μl x n samples
Carrier Mix	0.2 ml	1.2 ml	12.5 μl x n samples
Final volume	4.2 ml	25.2 ml	262.5 μl x n samples

- 5. Transfer **200** μ**l** Lysis Solution V / Carrier Mix directly into the <u>third</u> <u>cavity</u> of the Reagent Plate.
- Transfer 200 μl of the cleared supernatant from step 3 to the <u>third</u> <u>cavity</u> of the Reagent Plate containing Lysis Solution V / Carrier Mix.
- 7. Add **20** µl Proteinase K to the third cavity of the Reagent Plate.
- 8. The sample will be processed using the InnuPure C16 / C16 touch. Please follow the instructions of chapter 13 p. 21.

12.7 <u>Protocol 7</u>: Isolation from 200 μl of stool samples (e.g. Norovirus extraction) for subsequent detection by ELISA

NOTE

This protocol has to be used if the initial fecal sample is mixed with special buffers for subsequent detection by ELISA.

- 1. Transfer **25 μl** of **MAG Suspension** directly into the liquid of the <u>first</u> <u>cavity</u> of the Reagent Plate.
- 2. Transfer **250** µl of the sample into a 1.5 ml reaction tube and centrifuge the tube at max. speed for 3 minutes.
- 3. Prepare the mixture of Lysis Solution V and Carrier Mix according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	4.0 ml	24.0 ml	250 μl x n samples
Carrier Mix	0.2 ml	1.2 ml	12.5 μl x n samples
Final volume	4.2 ml	25.2 ml	262.5 μl x n samples

- 4. Transfer 200 μl Lysis Solution V / Carrier Mix directly into the <u>third</u> <u>cavity</u> of the Reagent Plate.
- Transfer 200 μI of the cleared supernatant from step 2 to the third cavity of the Reagent Plate containing
 Lysis Solution V / Carrier Mix.
- 6. Add **20** μl Proteinase K to the third cavity of the Reagent Plate.
- 7. The sample will be processed using the InnuPure C16 / C16 touch. Please follow the instructions of chapter 13 p. 21.

12.8 Protocol 8: Isolation from 20 mg of shrimp sample

- 1. Transfer **25** µl of **MAG Suspension** directly into the liquid of the <u>first</u> cavity of the Reagent Plate.
- 2. Transfer the sample into a Lysis Tube P (not included in the kit) and add 400 μl ddH₂O (DNase-, RNase-free). Homogenize the sample using SpeedMill PLUS (Analytik Jena AG).

NOTE

It is important to homogenize the sample completely! Do not use more than 20 mg of shrimp tissue. The time for homogenizing must be determined individually. We recommend starting with 1 minute homogenization time.

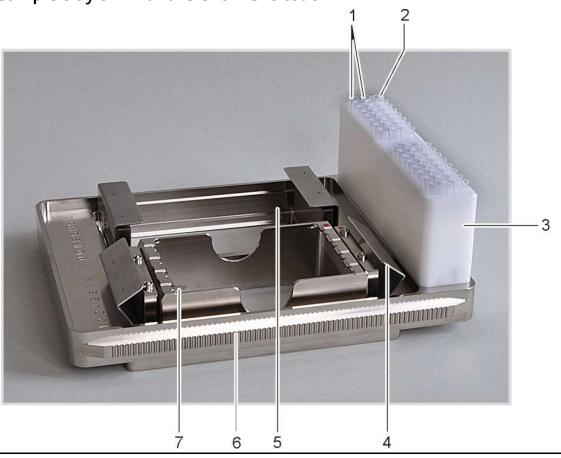
- 3. After homogenization centrifuge the Lysis Tube P at max. speed for 2 minutes. Use only **200** μ l of cleared supernatant for the following steps.
- 4. Prepare the mixture of Lysis Solution V and Carrier Mix according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	4.0 ml	24.0 ml	250 μl x n samples
Carrier Mix	0.2 ml	1.2 ml	12.5 μl x n samples
Final volume	4.2 ml	25.2 ml	262.5 µl x n samples

- 5. Transfer **200** μ**l** Lysis Solution V / Carrier Mix directly into the <u>third</u> <u>cavity</u> of the Reagent Plate.
- 6. Transfer **200** μ**I** of the sample from step 3 to the <u>third cavity</u> of the Reagent Plate containing Lysis Solution V / Carrier Mix.
- 7. Add **20** µl Proteinase K to the third cavity of the Reagent Plate.
- 8. The sample will be processed using the InnuPure C16 / C16 touch. Please follow the instructions of chapter 13 p. 21.

13 Automated extraction using InnuPure C16 / C16 touch

13.1 Sample tray of InnuPure C16 / C16 touch



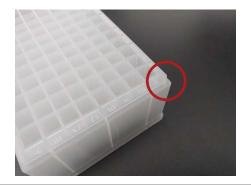
No. 1:	Filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Holding-down clamp
No. 5:	Sample block for Reagent Plates
No. 6:	Serrated guide rail (C16 touch: non-serrated)
No. 7:	Adapter for Reagent Strips

13.2 Preparing the 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 clamps 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 on the holder.

Reagent Plate

The notched corners of the Reagent Plate must align with 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 clamps 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 holes of the tip block.
- 5. Place the Elution Tubes into the wider holes 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 of up to 200 μ l use Elution Strips (0.2 ml). For high elution volumes of 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 Plates forward into the sample tray adapter 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 injury

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

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

Extraction procedure	Protocol on InnuPure®C16
Protocol 1 (Starting volume: 200 μl)	Int_Lysis_200_C16_04
Protocol 2 (Starting volume: 400 μl)	Int_Lysis_400_C16_04
Protocol 3 (Starting volume: 600 μl)	Int_Lysis_600_C16_04
Protocol 4, 5, 6, 7, 8 (Starting volume: 200 μl)	Int_Lysis_200_C16_04 Int_Lysis_200_FAST_C16_04 Int_Lysis_200_Ultra_FAST_C16_04

4. Enter the recommended elution volume of 100 μ l and press [OK].

NOTE

It is possible to adjust the elution volume values from 20 μ l to 500 μ l.

5. If needed, choose log file and enter sample IDs, press [OK] or [CANCEL].

NOTF

It is possible to enter sample IDs and to create a run log file. Find detailed information on 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]. The sample tray will be moved out of the device.

NOTE

The chosen protocol is performed by the device. After the protocol is finished, the tray with the purified samples will be moved out of the device upon pressing [NEXT]. The message "Program finished" will be displayed 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.
- After finishing the extraction protocol, the Elution Tubes contain the extracted DNA or RNA. Close the lids and store the samples under proper conditions.

NOTE

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

13.4 Starting the InnuPure C16 touch

NOTE

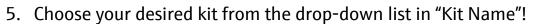
The following instructions describe the necessary steps for the start of the InnuPure C16 touch. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 touch.

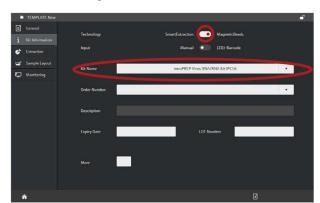
1. Switch on the InnuPure C16 *touch*. Wait until the home screen of IPextract is displayed on the tablet screen.



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"!



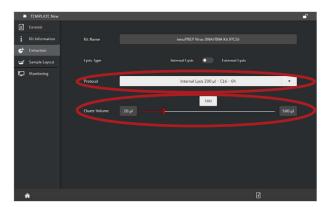


NOTE "Kit Information" tab

- 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 (Starting volume: 200 μl)	Internal Lysis 200 μl – C16 – 05
Protocol 2 (Starting volume: 400 μl)	Internal Lysis 400 μl – C16 – 05
Protocol 3 (Starting volume: 600 μl)	Internal Lysis 600 μl – C16 – 05
Protocol 4, 5, 6, 7, 8 (Starting volume: 200 μl)	Internal Lysis 200 µl – C16 – 05 Internal Lysis 200 µl – Fast – C16 – 05 Internal Lysis 200 µl – Ultra Fast – C16 – 05

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

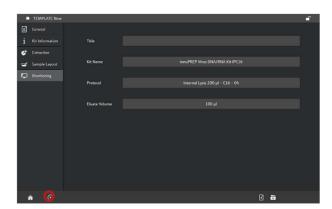


NOTE

"Extraction" tab

The recommended elution volume is $100 \, \mu l$.

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. After loading the tray into the device, a message appears reminding you that all cavities must be open before starting. If you have closed the Reagent Plates with a foil, please remove it. Please ignore the message if you have not sealed the Reagent Plates. The message must still be confirmed for the protocol to start.
- 12. 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.
- 13. The Elution Tubes contain the extracted DNA or RNA. Close the lids and store the DNA under proper conditions.

NOTE

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

14 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted viral RNA/DNA	A
Content of viral nucleic acid in sample insufficient.	Use more starting material, e.g. use 400 µl instead of 200 µl sample. Ensure to choose the appropriate extraction protocol.
Insufficient lysis of starting material.	Ensure to use the required volume of Proteinase K for current protocols, e.g. 20 µl Proteinase K for 200 µl of sample, but 30 µl Proteinase K for 400 µl of sample.
Eluate volume too high.	Decrease the eluate volume. The suggested eluate volume is 100 µl. Please note that lowering the eluate volume will not necessarily increase the yield proportionally!
Inadequate extraction.	Inhibiting substances in starting material. Please use the kit only for samples that match the requirements declared in "Product specifications".
	Use Internal Controls for verification of extraction procedure.

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