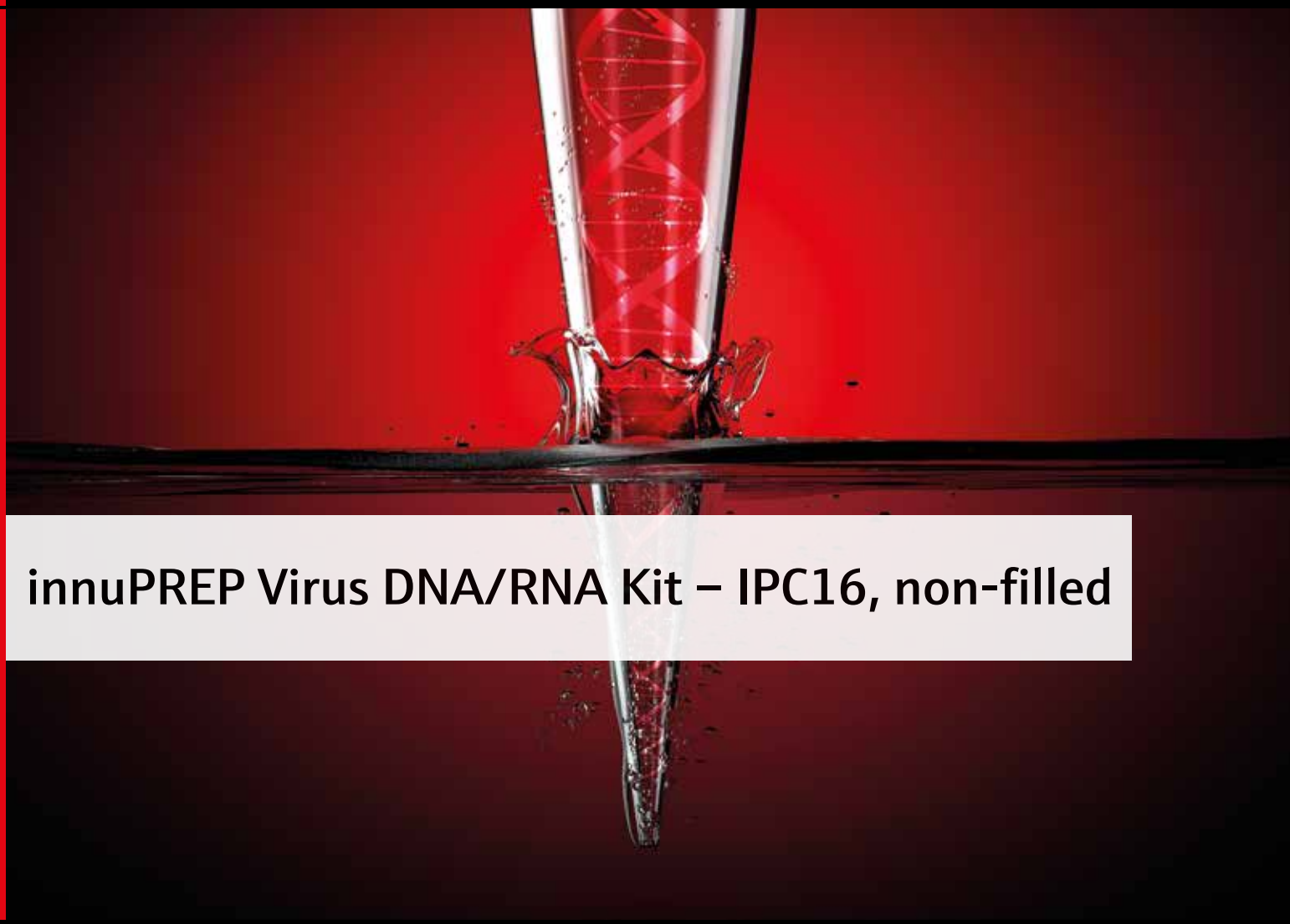
# Instructions for Use
Life Science Kits & Assays



# innuPREP Virus DNA/RNA Kit – IPC16, non-filled

analytikjena
An Endress+Hauser Company

**Order No.:**
845-PPP-7116016 16 reactions
845-PPP-7116096 96 reactions
845-PPP-7116480 480 reactions

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# Contents

# 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 µl, 400 µl and 600 µl of serum, plasma and other cell free samples. Furthermore, 200 µl 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.

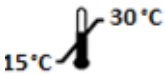
For the liquid samples all steps of the extraction process are fully automated and run completely on the InnuPure C16 / C16 *touch*. The samples are transferred into the Reagent Plates of the kit, which must be prefilled with all reagents needed for the
extraction procedure. The extraction process runs 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 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**<br>Catalogue number. |
|  N | **Content**<br>Contains sufficient reagents for <N> tests. |
|  | **Storage conditions**<br>Store at room temperature, unless otherwise specified. |
|  | **Consult instructions for use**<br>This information must be observed to avoid improper use of the kit and the kit components. |
|  | **Expiry date** |
|  | **Lot number**<br>The number of the kit charge. |
|  | **Manufactured by**<br>Contact information of manufacturer. |
|  | **For single use only**<br>Do not use components for a second time. |
| | **Note / Attention**<br>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 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.

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 the GHS classification please download the Safety Data Sheet (SDS) from our website (www.analytik-jena.com). Find the SDS of each product and its components in the “Downloads”section.

# 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 **Proteinase K** at 4 °C to 8 °C! Store dissolved **Proteinase K** in aliquots at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!

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 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

Before every use make sure that all components are at room temperature. If there are any precipitates within the provided solutions, they can be dissolved by careful warming.

# 5 Functional testing and technical assistance

The Analytik Jena AG 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 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 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 Analytik Jena AG kits have only been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits when 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**
The kit is for research use only!

# 7 Kit components

## 7.1 Components included in the kit

| | Σ 16 | Σ 96 | Σ 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 | 2 x 160 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 | 4 x 200 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
- dd$H_2O$ for dissolving **Proteinase K**
- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$) for Protocol 6
- Physiological saline (0,9 % NaCl) for Protocol 5
- Lysis Tubes P (innuSPEED Lysis Tube P, 845-CS-1020250) for Protocol 8

### 7.3 Related Products

- Deep Well Plate (96 square well, 2.0 ml 845-FX-8500025, 25 pcs)

## 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 $ddH_2O$ 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 µl |
| | 7 | Washing Solution A | 600 µl |
| | 8 | Washing Solution A | 600 µl |
| | 9 | Washing Solution B2 | 600 µl |
| | 10 | Washing Solution B2 | 600 µl |
| | 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 prior to use (approx. 30 seconds)!

## 12.1 Protocol 1: Isolation from 200 µl cell-free body fluids (serum, plasma, cerebrospinal fluid, liquor)

1. Transfer **25 µl** of **MAG Suspension** directly into the liquid of the **first 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** | 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 **third cavity** 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 µl** of **MAG Suspension** directly into the liquid of the **first 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 **third cavity** of the Reagent Plate.
4. Add **400 µl** of the sample to the **third cavity** 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 **first 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 **third cavity** of the Reagent Plate.

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

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

1. Transfer **25 µl** of **MAG Suspension** directly into the liquid of the **first cavity** 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 **third cavity** 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.

## 12.5 Protocol 5: 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 **first cavity** 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 **third cavity** of the Reagent Plate.
5. Transfer **200 µl** of the sample 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.

## 12.6 Protocol 6: 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 **first cavity** of the Reagent Plate.
2. Transfer about 50–100 mg of the stool sample into a 1.5 ml reaction tube and add 250 µl 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 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Plate.
6. Transfer **200 µl** of the cleared supernatant from step 3 to the **third cavity** 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 Protocol 7: 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 **first cavity** 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 **third cavity** of the Reagent Plate.
5. Transfer **200 µl** 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 **first cavity** of the Reagent Plate.
2. Transfer the sample into a **Lysis Tube P** (not included in the kit) and add **400 µl dd$H_2O$** (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.

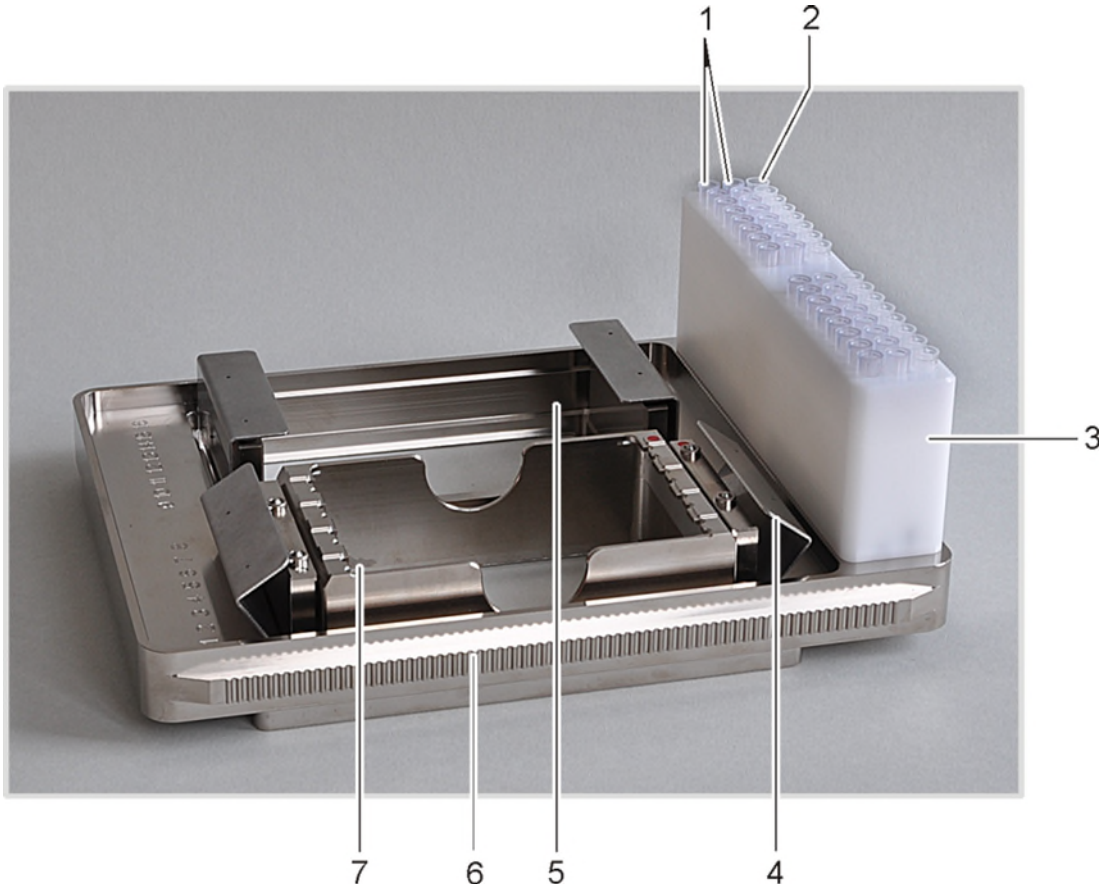
3. After homogenization centrifuge the Lysis Tube P at max. speed for 2 minutes. Use only **200 µl** of **cleared supernatant** for the following steps.
4. Prepare the mixture of **Lysis Solution V** and **Carrier Mix** according to the table below.

| 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 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Plate.
6. Transfer **200 µl** of the sample from step 3 to the **third cavity** 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 |

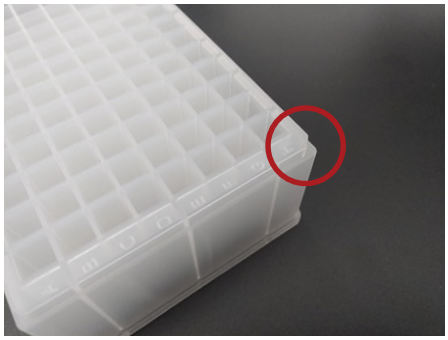
## 13.2 Preparing the sample tray of InnuPure C16 / C16 *touch*

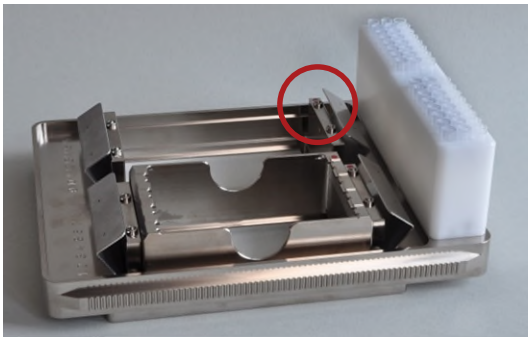
1. Place the InnuPure C16 / C16 *touch* sample tray into the priming station and open the holding-down clamps of the sample tray!

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**

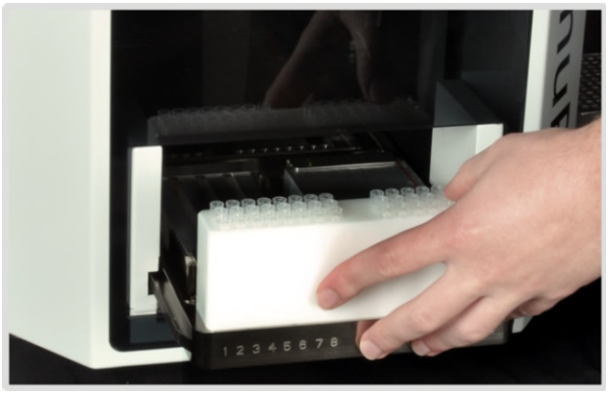
Make sure that for every sample the tips and the elution vessel are in the corresponding positions in the tip block!

**IMPORTANT NOTE**

It is possible to select between two different elution vessels! For small elution volumes of up to 200 µl use Elution Strips (0.2 ml). For high elution volumes of 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 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**<br>(Starting volume: 200 µl) | Int_Lysis_200_C16_04 |
| **Protocol 2**<br>(Starting volume: 400 µl) | Int_Lysis_400_C16_04 |
| **Protocol 3**<br>(Starting volume: 600 µl) | Int_Lysis_600_C16_04 |
| **Protocol 4, 5, 6, 7, 8**<br>(Starting volume: 200 µl) | Int_Lysis_200_C16_04<br>Int_Lysis_200_FAST_C16_04<br>Int_Lysis_200_Ultra_FAST_C16_04 |

4. Enter the recommended **elution volume** of **100 µl** and press [OK].

**NOTE**
It is possible to adjust the elution volume values from 20 µl to 500 µl.

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

**NOTE**
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!

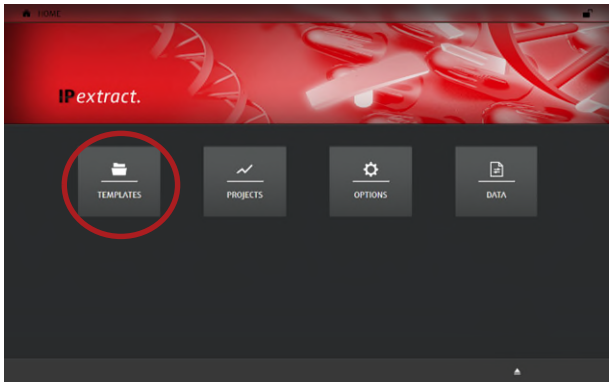
7. Remove the sample tray from the adapter of the InnuPure C16 and place it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes contain the extracted DNA or RNA. Close the lids and store the samples under proper conditions.

**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*.

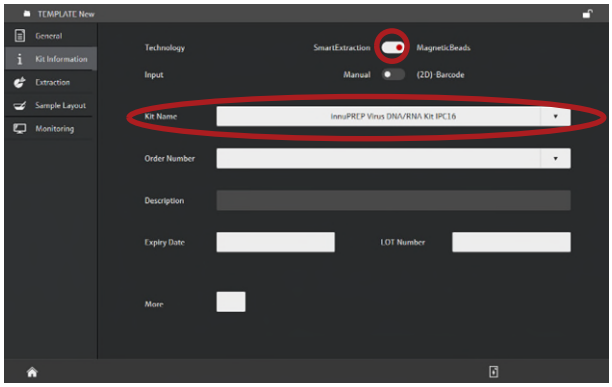
1. Switch on the InnuPure C16 *touch*. Wait until the home screen of IP*extract* is displayed on the tablet screen.




**NOTE**
Home screen of IP*extract*

2. Choose [TEMPLATES]→[New Template]→[Kit-based].
3. Enter optional information in the tab “General”.
4. Choose the tab “Kit Information” and switch the “Technology” to “MagneticBeads”!

5. Choose your desired kit from the drop-down list in “Kit Name”!

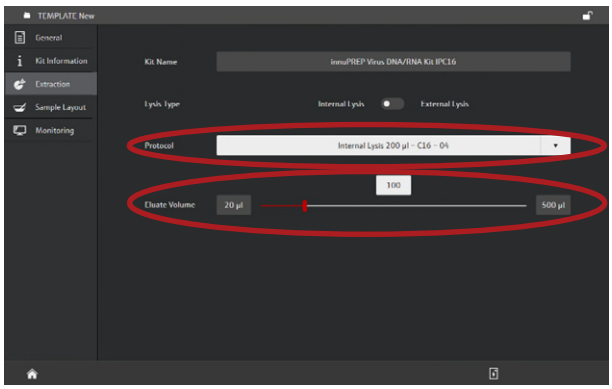



**NOTE**
“Kit Information” tab

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

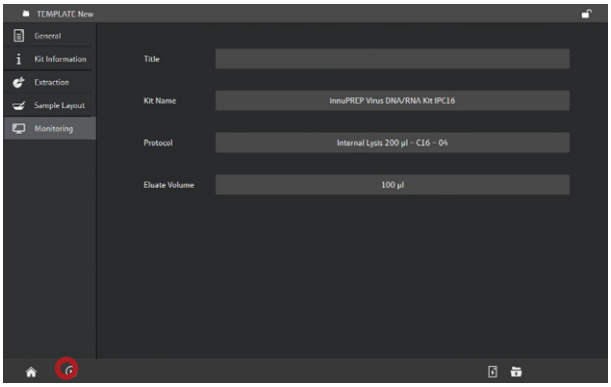
8. Adjust the “Eluate Volume“ using the slider or the text field.




**NOTE**
“Extraction” tab

The recommended elution volume is 100 µl.

9. Choose the tab “Monitoring” and start the protocol by tapping the start button.




**NOTE**
“Monitoring” tab

10. Follow the instructions displayed on the tablet screen.

11. 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** | |
| 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”.<br><br>Use Internal Controls for verification of extraction procedure. |

en 08 / 20 – Analytik Jena AG, Jena

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