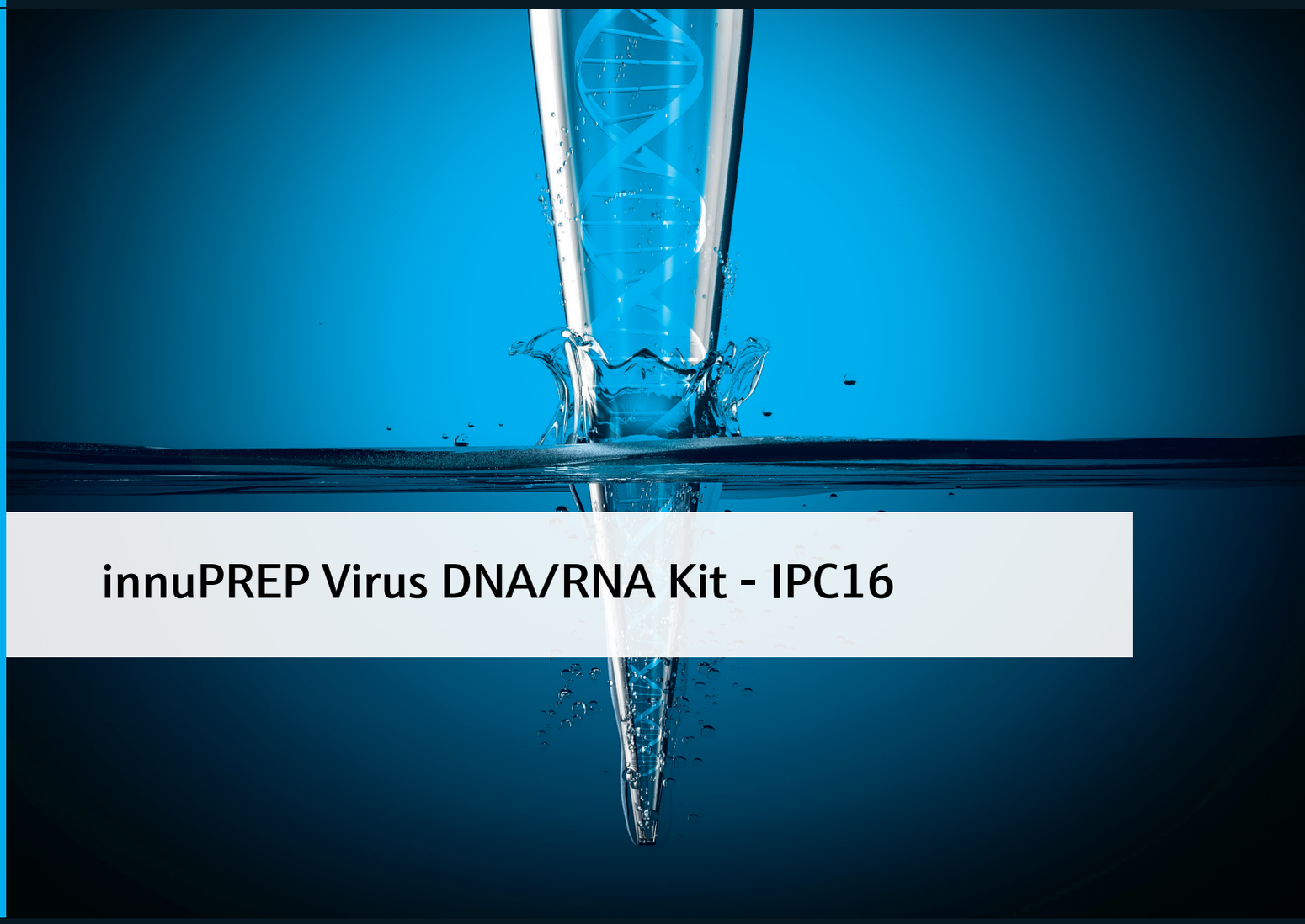
Instructions for Use
Life Science Kits & Assays



innuPREP Virus DNA/RNA Kit - IPC16

Innuscreen GmbH

Order No.:

845-IPS-7016016 16 reactions
845-IPS-7016096 96 reactions
845-IPP-7016016 16 reactions
845-IPP-7016096 96 reactions
845-IPP-7016480 480 reactions

Publication No.: HB_IP_7016_e_230401

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Contents

1 Introduction

1.1 Intended use

The **innuPREP Virus DNA/RNA Kit – IPC16** 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 *touch*. The samples are transferred into the Reagent Strips or Reagent Plates of the kit, which are already prefilled with all extraction reagents needed for the extraction process. The following extraction process runs automatically on the InnuPure C16 *touch*. The extraction process is based on binding of the DNA and/or RNA to 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 *touch* protocol are optimized to get maximum yield and quality.

Further, the kit contains a Carrier Mix with a Carrier RNA and an Internal Control DNA (IC DNA) for controlling the extraction process and for better recovery of minute amounts of sample DNA. The IC DNA can be detected by Real-time PCR with a corresponding Real-time PCR detection kit.

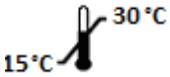
Please note that the eluates of the kit contain both sample DNA and Carrier Mix. Therefore, it is not possible to quantify the isolated nucleic acids by photometric or fluorometric methods when using the Carrier Mix. Thus other methods for quantification such as specific quantitative PCR or Real-time PCR systems are recommended. Furthermore, Carrier RNA may inhibit PCR reactions. The amount of added Carrier RNA may thus be carefully optimized depending on the individual PCR system used.

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

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. 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, 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 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 potential infectious human 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.
- 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

The kit is shipped at ambient temperature.

Store lyophilized and dissolved **Proteinase K and MAG Suspension** at 4 °C to 8 °C.

Store lyophilized and dissolved **Carrier Mix** at -22 °C to -18 °C. Aliquot dissolved **Carrier Mix** and do not freeze and thaw it more than 3 times!

The set up 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 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 Virus DNA/RNA Kit – IPC16, please do not hesitate to contact us.

For technical support or further information in Germany please contact info.innu@ist-ag.com.

6 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ "Intended use" p. 3) (→ "Product specifications" p. 13). 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 Components included in the kit

	Σ 16	Σ 96	Σ 480
REF	845-IPS-7016016[a] 845-IPP-7016016[b]	845-IPS-7016096[a] 845-IPP-7016096[b]	845-IPP-7016480[b]
MAG Suspension	1 ml	2 x 2 ml	2 x 9 ml
Lysis Solution V	15 ml	2 x 60 ml	2 x 230 ml
Proteinase K	For 1.5 ml working solution	For 4 x 1.5 ml working solution	For 16 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
RNase-free Water	2 ml	2 ml	5 x 2 ml
Reagent Strip O[a]	16 (pre-filled, sealed)	96 (pre-filled, sealed)	--
Reagent Plate O[b]	2 (pre-filled, sealed)	12 (pre-filled, sealed)	60 (pre-filled, sealed)
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

- 15 ml tubes
- 1.5 ml tubes
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- Physiological saline (0.9 % NaCl)
- ddH_2O for dissolving **Proteinase K**
- Piercing Tool, (12 well Piercer) 845-PTS-0000005, IST Innuscreen GmbH, Germany
- Lysis Tube P (innuSPEED Lysis Tube P, 845-CS-1020250, IST Innuscreen GmbH, Germany)

8 Initial steps before starting

- Add 1.5 ml ddH_2O to lyophilized **Proteinase K,** mix thoroughly and store as described above.
- Add 1.25 ml RNase-free Water to lyophilized **Carrier Mix,** mix thoroughly and store as described above.
- Prepare **Lysis Solution V / Carrier Mix** as indicated in each protocol.
- Avoid freezing and thawing of starting material.
- 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.
- Vortex MAG Suspension for 30 seconds

9 Usage of Internal Controls

Besides carrier RNA the **Carrier Mix** contains an internal control DNA and RNA (IC DNA and IC RNA). Both can be detected by real-time PCR using the corresponding assays.

If customized extraction controls are used, please add these components to the mixture of **Lysis Solution V** and **Carrier Mix** (→ “Protocols” p. 15).

10 Product specifications

1. Starting material:

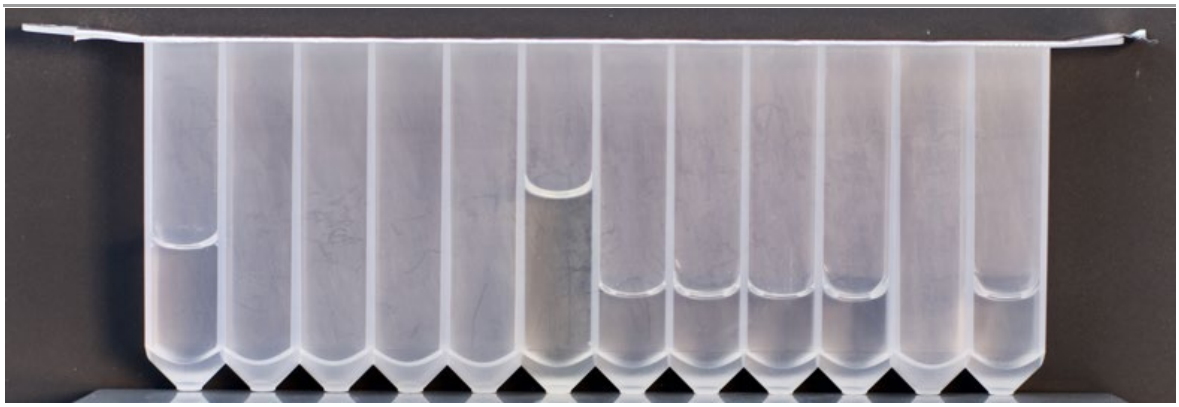
- Cell-free body fluids (serum, plasma, cerebrospinal fluid, liquor); 200 µl, 400 µl or 600 µl sample volume.
- Cell culture supernatants; max. 200 µl sample volume.
- Swab samples; max. 200 µl sample volume.
- Stool samples; max. 200 µl sample volume.

2. Time for isolation:

Extraction protocol	Protocol on InnuPure C16 touch	Time InnuPure C16 touch	Elution volumes
Internal Lysis 200 µl – C16 – 05	200 µl	77 min	20–500 µl
Internal Lysis 200 µl – Fast – C16 – 05	200 µl	58 min	20–500 µl
Internal Lysis 200 µl – Ultra Fast – C16 – 05	200 µl	31 min	20–500 µl
Internal Lysis 400 µl – C16 – 05	400 µl	89 min	20–500 µl
Internal Lysis 600 µl – C16 – 05	600 µl	97 min	20–500 µl

11 Preparing Reagent Plate / Strip for automated extraction

11.1 General filling scheme of reagent reservoir



Cavity 1:	RNase-free Water	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 Strip

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

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

Carefully open the overpack of Reagent Plates by using scissors.

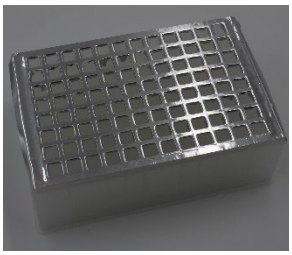
11.3 Piercing of sealing foil of Reagent Plate or Reagent Strip

NOTE
Before using Reagent Plates or Strips the sealing foil has to be pierced manually. Always wear gloves while piercing of the foil!

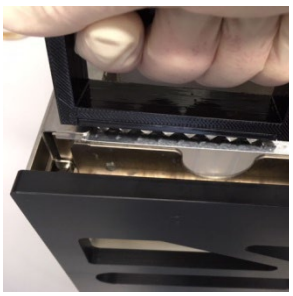
Reagent Plates or Strips are prefilled with extraction reagents and are sealed with a foil. This foil has to be pierced manually before use, by using the piercing tools (12 well piercer).
Keep the Reagent Plates or Strips in a horizontal position to avoid spilling of the reagents while piercing of the foil.
Open all cavities (one row per sample).

Using plates



Using Reagent Strips




12 Protocols for isolation of viral DNA and RNA

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

NOTE
The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.
Ensure the **Carrier Mix** has been prepared as described (→ "Kit components" p. 10) and Reagent Plates / Strips are pierced (→ „Preparing Reagent Plate or Reagent Strip for automated extraction" p. 14).

1. Prepare 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

2. Transfer **25 µl of MAG Suspension** directly into the liquid of the first cavity of the Reagent Plate / Strip.
3. Transfer **200 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of Reagent Strip or Reagent Plate.
4. Add **200 µl** of the sample to the **third cavity** of the Reagent Strip or Reagent Plate containing **Lysis Solution V / Carrier Mix**.
5. Add **20 µl Proteinase K** to the **third cavity** of the Reagent Strip or Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 13 p. 24.

12.2 Protocol 2: Isolation from 400 µl cell-free body fluids (serum, plasma, cerebrospinal fluid, liquor)

NOTE
The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.
Ensure the **Carrier Mix** has been prepared as described (→ "Kit components" p. 10) and Reagent Plates / Strips are pierced (→ „Preparing Reagent Plate or Reagent Strip for automated extraction" p. 14).

1. Prepare mixture of **Lysis Solution V** and **Carrier Mix** according to the table below.

Component	16 samples	96 samples	n samples
Lysis Solution V	8.0 ml	48.0 ml	500 µl x n samples
Carrier Mix	0.2 ml	1.2 ml	12.5 µl x n samples
Final volume	8.2 ml	49.2 ml	512.5 µl x n samples

2. Transfer **25 µl of MAG Suspension** directly into the liquid of the first cavity of the Reagent Plate / Strip.
3. Transfer **400 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Strip or 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 Strip or Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 13 p. 24.

12.3 Protocol 3: Isolation from 600 µl cell-free body fluids (serum, plasma, cerebrospinal fluid, liquor)

NOTE
The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.
Ensure the **Carrier Mix** has been prepared as described (→ "Kit components" p. 10). and Reagent Plates / Strips are pierced (→ „Preparing Reagent Plate or Reagent Strip for automated extraction" p. 14).

1. Prepare mixture of **Lysis Solution V** and **Carrier Mix** according to the table below.

Component	16 samples	96 samples	n x samples
Lysis Solution V	12.0 ml	72.0 ml	750 µl x n samples
Carrier Mix	0.2 ml	1.2 ml	12.5 µl x n samples
Final volume	12.2 ml	73.2 ml	762.5 µl x n samples

2. Transfer **25 µl of MAG Suspension** directly into the liquid of the first cavity of the Reagent Plate / Strip.
3. Transfer **600 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Strip or Reagent Plate.
4. Add **600 µl** of the sample to the **third cavity** of the Reagent Strip or Reagent Plate containing **Lysis Solution V / Carrier Mix**.
5. Add **50 µl Proteinase K** to the **third cavity** of the Reagent Strip or Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 13 p. 24.

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

NOTE
The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.
Ensure the **Carrier Mix** has been prepared as described (→ "Kit components" p. 10). and Reagent Plates / Strips are pierced (→ „Preparing Reagent Plate or Reagent Strip for automated extraction" p. 14).

1. Prepare 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

2. Transfer **25 µl of MAG Suspension** directly into the liquid of the first cavity of the Reagent Plate / Strip.
3. Transfer **200 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Strip or Reagent Plate.
4. Add **200 µl** of the sample to the **third cavity** of the Reagent Strip or Reagent Plate containing **Lysis Solution V / Carrier Mix**.
5. Add **20 µl Proteinase K** to the **third cavity** of the Reagent Strip or Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 13 p. 24.

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

NOTE
The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.
Ensure the **Carrier Mix** has been prepared as described (→ "Kit components" p. 10) and Reagent Plates / Strips are pierced (→ „Preparing Reagent Plate or Reagent Strip for automated extraction" p. 14).

1. Place the swabs into 1.5 ml reaction tubes containing **500 µl physiological saline**, incubate for 10 minutes and shake the swab vigorously inside the solution, squeeze it at the wall of the tube and remove the swab.
2. Prepare 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 **25 µl of MAG Suspension** directly into the liquid of the first cavity of the Reagent Plate / Strip.
4. Transfer **200 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Strip or Reagent Plate.
5. Transfer **200 µl** of the sample to the **third cavity** of the Reagent Strip or Reagent Plate containing **Lysis Solution V / Carrier Mix**.
6. Add **20 µl Proteinase K** to the **third cavity** of the Reagent Strip or Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 13 p. 24.

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

NOTE

The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.
Ensure the **Carrier Mix** has been prepared as described (→ "Kit components" p. 10) and Reagent Plates / Strips are pierced (→ „Preparing Reagent Plate or Reagent Strip for automated extraction" p. 14).

1. Prepare 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

2. Transfer **25 µl of MAG Suspension** directly into the liquid of the first cavity of the Reagent Plate / Strip.
3. Transfer about 0.05–0.1 g of the stool sample into a 1.5 ml reaction tube and add 250 µl PBS.
4. Vortex the sample for 5 seconds and centrifuge it at max. speed for 3 minutes.
5. Transfer **200 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Strip or Reagent Plate.
6. Transfer **200 µl** of the cleared supernatant from step 3 to the **third cavity** of the Reagent Strip or Reagent Plate containing **Lysis Solution V / Carrier Mix**.
7. Add **20 µl Proteinase K** to the **third cavity** of the Reagent Strip or Reagent Plate.

NOTE

The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 13 p. 24.

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.
The lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.
Ensure the **Carrier Mix** has been prepared as described (→ "Kit components" p. 10) and Reagent Plates / Strips are pierced (→ „Preparing Reagent Plate or Reagent Strip for automated extraction" p. 14).

1. Prepare 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

2. Transfer **25 µl of MAG Suspension** directly into the liquid of the first cavity of the Reagent Plate / Strip.
3. Transfer **250 µl of the sample** into a 1.5 ml reaction tube and centrifuge the tube at max. speed for 3 minutes.
4. Transfer **200 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Strip or Reagent Plate.
5. Transfer **200 µl** of the **cleared supernatant** from step 2 to the **third cavity** of the Reagent Strip or Reagent Plate containing **Lysis Solution V / Carrier Mix**.
6. Add **20 µl Proteinase K** to the **third cavity** of the Reagent Strip or Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 13 p. 24.

12.8 Protocol 8: Isolation from 20 mg shrimp sample

NOTE
Do not use more than 20 mg of shrimp tissue. The homogenization has to be done by the user; the lysis of the starting material is done automatically and is included in the InnuPure C16 *touch* extraction protocol.
Ensure the **Carrier Mix** has been prepared as described (→ "Kit components" p. 10) and Reagent Plates or Reagent Strips (→ „Preparing Reagent Plate / Strip for automated extraction" p. 14).

1. Prepare mixture of **Lysis Solution V** and **Carrier Mix** according to the table below.

Component	16 samples	96 samples	n x 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

2. Transfer the sample into a **Lysis Tube P** (not included in the kit) and add **400 µl ddH_2O** (DNase-, RNase-free). Homogenize the sample using SpeedMill PLUS (Analytik Jena AG).

NOTE
It is important to homogenize the sample completely! The time for homogenizing must be determined individually. We recommend starting with 1 minute homogenization time.

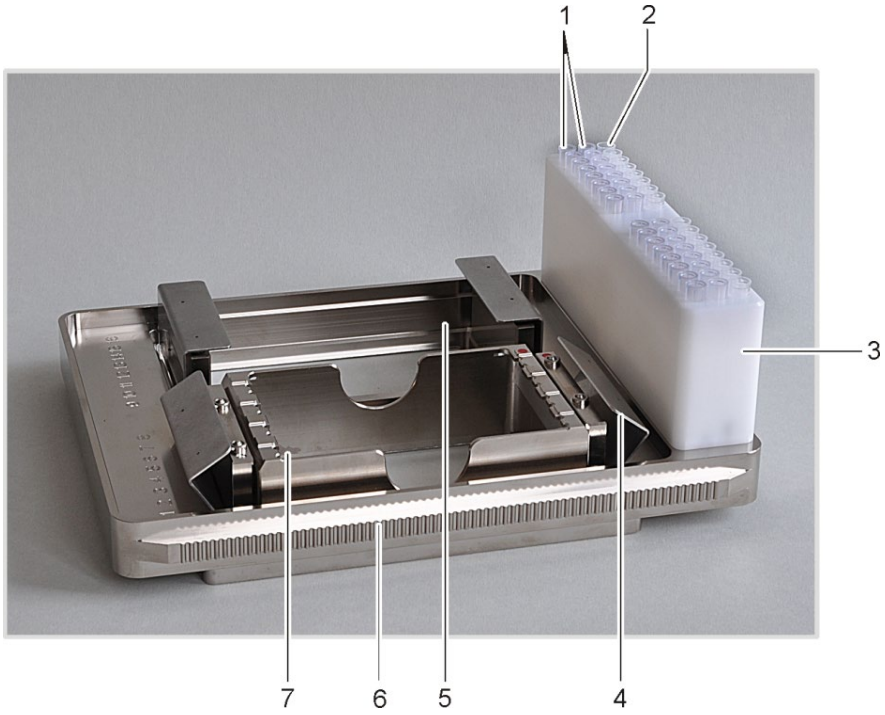
3. After homogenization centrifuge the Lysis Tube P at max. speed for 2 minutes. Use only **200 µl** of **cleared supernatant** for the following steps.
4. Transfer **25 µl of MAG Suspension** directly into the liquid of the first cavity of the Reagent Plate / Strip.
5. Transfer **200 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Strip or Reagent Plate.

6. Transfer **200 µl** of the sample from step 3 to the **third cavity** of the Reagent Strip or Reagent Plate containing **Lysis Solution V / Carrier Mix**.
7. Add **20 µl Proteinase K** to the **third cavity** of the Reagent Strip or Reagent Plate.

NOTE
The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 13 p. 24.

13 Automated extraction using InnuPure C16 touch

13.1 Sample tray of InnuPure C16 touch



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

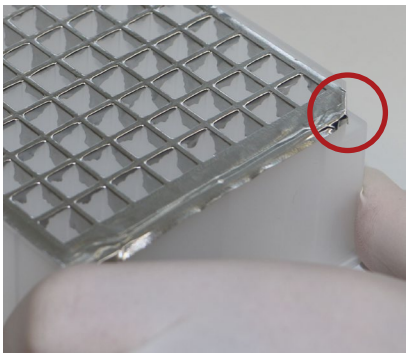
13.2 Preparing sample tray of InnuPure C16 touch

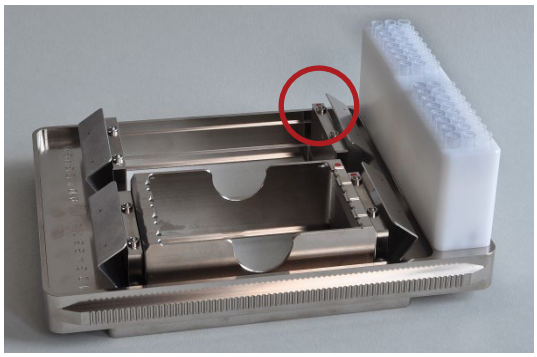
NOTE
The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more strips as number of samples!

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

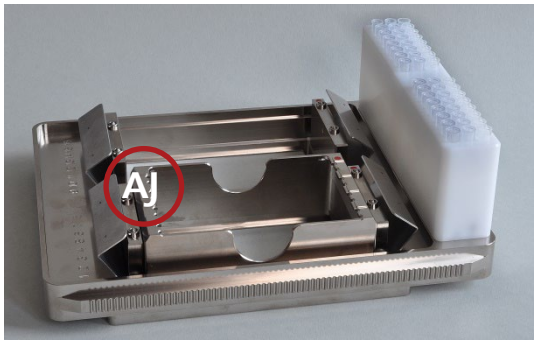
The notched corners of the Reagent Plate must point to the colored dot on the holder.





Reagent Strips

Place the Reagent Strips into the adapter. The long tab marked with the label "AJ" must point to the side of the adapter which is more distant from the tip block.



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

3. Fold down the holding-down clamp to prevent the Reagent Plates and Reagent Strips to be pulled out of the holder during the extraction process.
4. For each extracted sample place two filter tips in the smaller drill holes of the tip block.
5. Place the Elution Tubes into the wider drill hole at the edge of the tip block. Empty sample positions do not need to be filled.

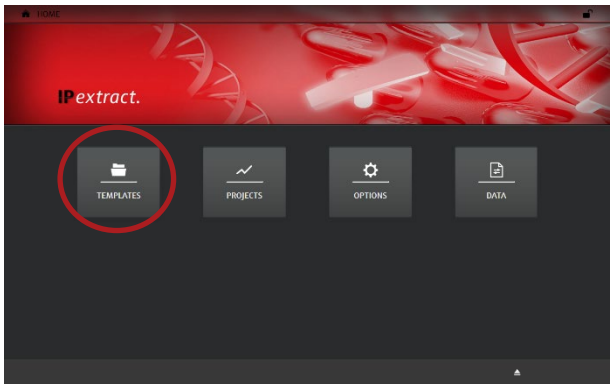
NOTE
Especially with the Reagent Strips make sure that for every Reagent Strip the tips and the elution vessel are in the corresponding positions in the tip block!

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 (Strips).

13.3 Starting the InnuPure C16 touch

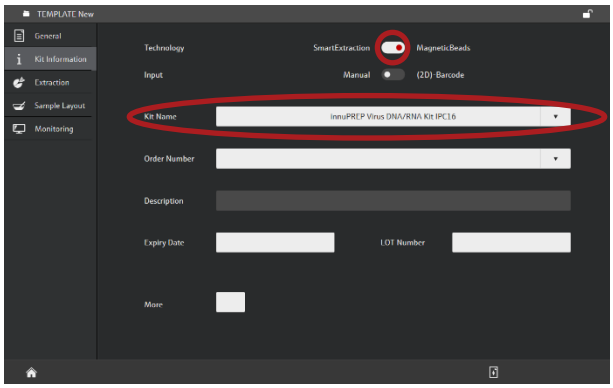
NOTE
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

1. Switch on the InnuPure C16 *touch* and the tablet computer. Wait until the home screen of 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 “Kit Name”!



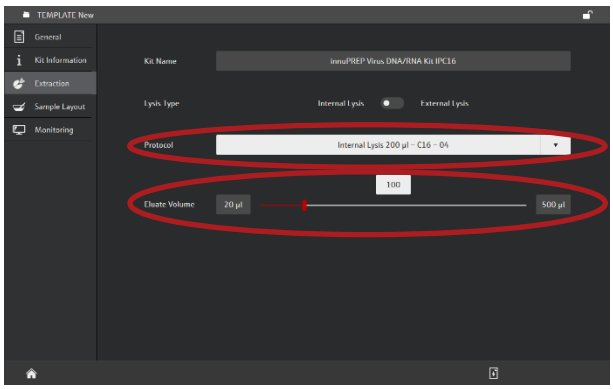
NOTE
“Kit Information” tab

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 (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

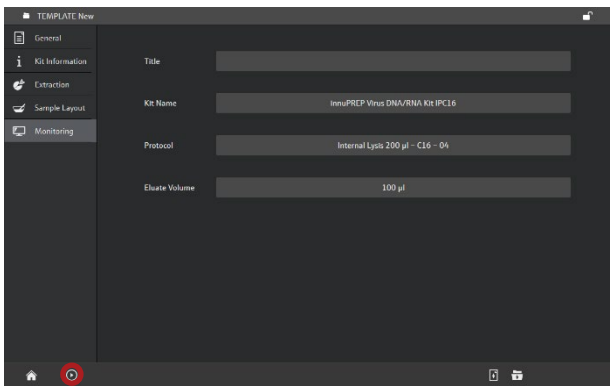
8. Adjust your desired “Eluate Volume“ using the slider or the text field.



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. Completion of the protocol is indicated by a message on the tablet screen. Follow the instructions on the screen to remove the sample tray from the device.
12. The Elution Tubes contain the extracted DNA 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	
No extracted DNA/RNA	No magnetic beads added to cavity 1. Please add 25 µl MAG Suspension to cavity 1 prior the extraction procedure. Ensure MAG Suspension has mixed well before use
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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