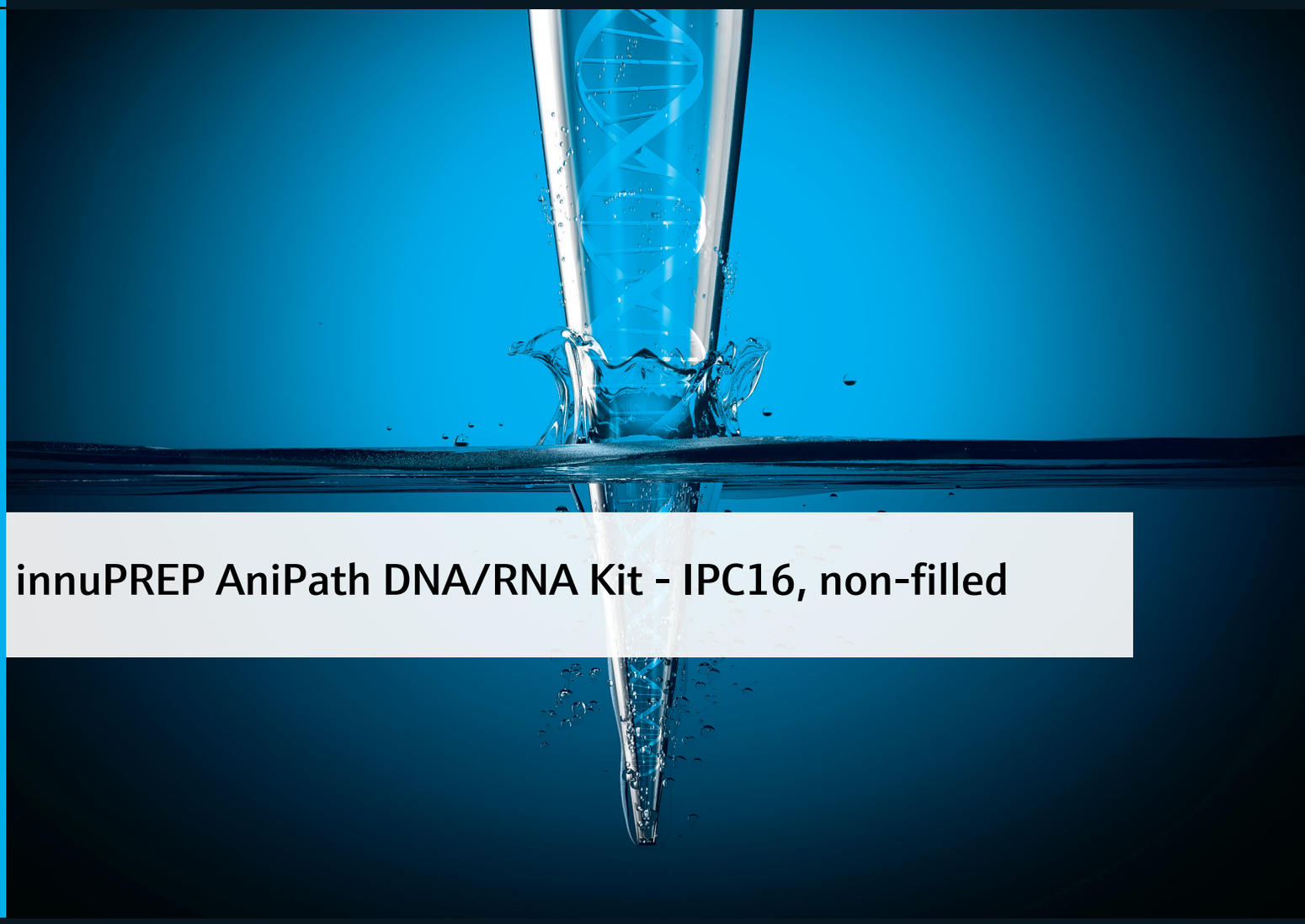
Instructions for Use

Life Science Kits & Assays



innuPREP AniPath DNA/RNA Kit - IPC16, non-filled

Innuscreen GmbH

Order No.:
845-PPP-8016016 16 reactions
845-PPP-8016096 96 reactions
845-PPP-8016480 480 reactions

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It needs not necessarily agree with future versions. Subject to change!

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1 Introduction

1.1 Intended use

The **innuPREP AniPath DNA/RNA Kit – IPC16, non-filled** has been designed for automated isolation of bacterial and viral DNA and RNA from different kinds of starting material like cell-free body fluids, cell culture supernatants or whole blood. Furthermore, 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 Plates of the kit, which must be prefilled with all reagents needed for the extraction process. The extraction procedure runs automatically on the InnuPure C16 *touch*. The extraction process 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 *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 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.
Σ N	**Content** Contains sufficient reagents for <N> tests.
15 °C 30 °C	**Storage conditions** Store at room temperature, unless otherwise specified.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual and the kit", p. 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 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 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 AniPath 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 should 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 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 AniPath 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 contact info.innu@ist-ag.com. 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 materials than those, referred to in the manual (→ “Product specifications”, p. 12). 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 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-PPP-8016016	845-PPP-8016096	845-PPP-8016480
MAG Suspension	1,5 ml	5,5 ml	3 x 9 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
Lysis Solution V	15 ml	120 ml	2 x 230 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	200 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	60
Elution Strips	2	12	60
Manual	1	1	1

7.2 Components not included in the kit

- 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**
- 96 %–99.8 % Ethanol (molecular biology grade, undenaturated)

7.3 Related Products

- Deep Well Plate (96 square well, 2.0 ml, 845-FX-8500025, 25 pcs)
- Deep Well Plate (96 square well, 2.0 ml, 845-FX-8500115, 115 pcs)
- IPC16 Dummy Plate (sealed, 31-00258, 1 piece)

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** (→ "Initial steps before starting" p. 13).

9 Product specifications

1. Starting material:

- Cell-free body fluids and cell culture supernatant (e.g. serum, plasma, cerebrospinal fluid; up to 400 µl)
- Whole blood samples (up to 400 µl)
- Swabs from nasopharyngeal samples (e.g. Influenza testing)
- Tissue samples (up to 10 mg)
- Stool samples (50–100 mg)

2. Time for isolation:

- Time required for external steps depends on the starting material
- Extraction on InnuPure C16 *touch*: 77 min

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. Always keep the bottle 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 below and store as described above.

Component	16 samples	96 samples	n samples
Lysis Solution V	8 ml	48 ml	500 µl x n samples
Carrier Mix	168 µl	1.0 ml	10.5 µl x n samples
Final volume	7.0 ml	42.3 ml	440.5 µl x n samples

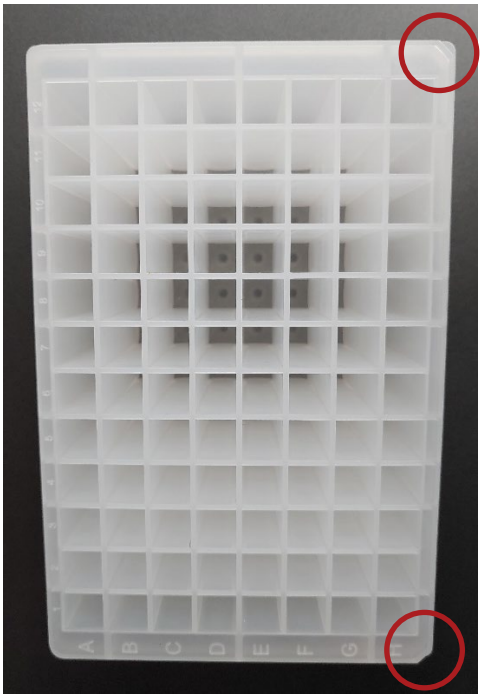
NOTE

The preparation of **Carrier Mix / Lysis Solution** mixture is not necessary for all sample types. Refer to the specific protocol used.

11 Prefilling Reagent Plate 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. Position 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 the indicated volume of the corresponding solution as specified in the table (e.g. fill each of the eight cavities of row 1 with 900 µl of RNase-free water) and also add **MAG Suspension**, **Sample** and **Proteinase K** as described in the chapter “Protocols”.

Deep Well Plate	Row No.	Solution	Volume per cavity
	1	RNase-free Water	900 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 or bacterial nucleic acids

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

NOTE
When working with cell free body fluids we recommend the addition of Carrier Mix. Ensure the **Carrier Mix** has been prepared as described (→ "Initial steps before starting", p. 13).
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)!

1. Transfer **50 µl of MAG Suspension** directly into the liquid of the **first cavity** of Reagent Plate.
2. Transfer **400 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Plate.
3. Add **200 µl PBS** to the **third cavity** of the Reagent Plate.
4. Add **200 µl** of the sample to the **third cavity** of the Reagent Plate.

NOTE
If the volume of the blood sample is less than 200 µl, adjust with PBS to 200 µl.

5. Add **50 µl Proteinase K** to the **third cavity** of the Reagent Plate.
6. The sample will be processed using the InnuPure C16 touch. Please follow the instructions of chapter 13 p. 21.

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

NOTE
When working with cell free body fluids we recommend the addition of Carrier Mix. Ensure the **Carrier Mix** has been prepared as described (→ "Initial steps before starting", p. 13).
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)!

1. Transfer **50 µl of MAG Suspension** directly into the liquid of the **first cavity** of Reagent Plate.
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 Plate.

NOTE
If the volume of the blood sample is less than 400 µl, adjust with PBS to 400 µl.

5. Add **50 µl Proteinase K** to the **third cavity** of the Reagent Plate.
6. The sample will be processed using the InnuPure C16 touch. Please follow the instructions of chapter 13 p. 21.

12.3 Protocol 3: Isolation from swabs from nasopharyngal samples (e.g. for Influenza testing)

NOTE
When working with nasopharyngal swab samples we recommend the addition of Carrier Mix. Ensure the **Carrier Mix** has been prepared as described (→ "Initial steps before starting", p. 13).
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)!

1. Transfer **50 µl of MAG Suspension** directly into the liquid of the **first cavity** of Reagent Plate.
2. Transfer **400 µl Lysis Solution V / Carrier Mix** directly into the **third cavity** of the Reagent Plate.
3. Place the swabs into 1.5 ml reaction tubes containing **500 µl physiological saline** (0.9 % NaCl, not included in the kit) and incubate for 20 minutes under constant shaking.
4. Squeeze the swab and remove the swab.
5. Add **400 µl** of the liquid sample to the **third cavity** of the Reagent Plate.
6. Add **50 µl Proteinase K** to the **third cavity** of the Reagent Plate.
7. The sample will be processed using the InnuPure C16 touch. Please follow the instructions of chapter 13 p. 21.

12.4 Protocol 4: Isolation from tissue homogenates

NOTE
Co-extraction of genomic nucleic acids can inhibit downstream PCR or real-time PCR applications!

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

1. Homogenize the tissue samples using bead based homogenizers (e.g. SpeedMill IST Innuscreen GmbH). For optimized results use 5-10 mg of tissue sample. Transfer the tissue sample into a homogenization tube and add **600 µl** dd$H_2$0 (RNase free) or PBS.
2. After homogenization centrifuge the sample at 10,000 x g for 2 minutes.
3. Transfer **50 µl of MAG Suspension** directly into the liquid of the **first cavity** of Reagent Plate.
4. Transfer **400 µl Lysis Solution V** into the **third cavity** of the Reagent Plate.
5. Add **400 µl** of the homogenized tissue sample to the **third cavity** of the Plate.
6. Add **50 µl Proteinase K** to the **third cavity** of the Reagent Plate.
7. The sample will be processed using the InnuPure C16 touch. Please follow the instructions of chapter 13 p. 21.

12.5 Protocol 5: Isolation from 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 Option 2.

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

12.5.1 Option 1: Standard procedure

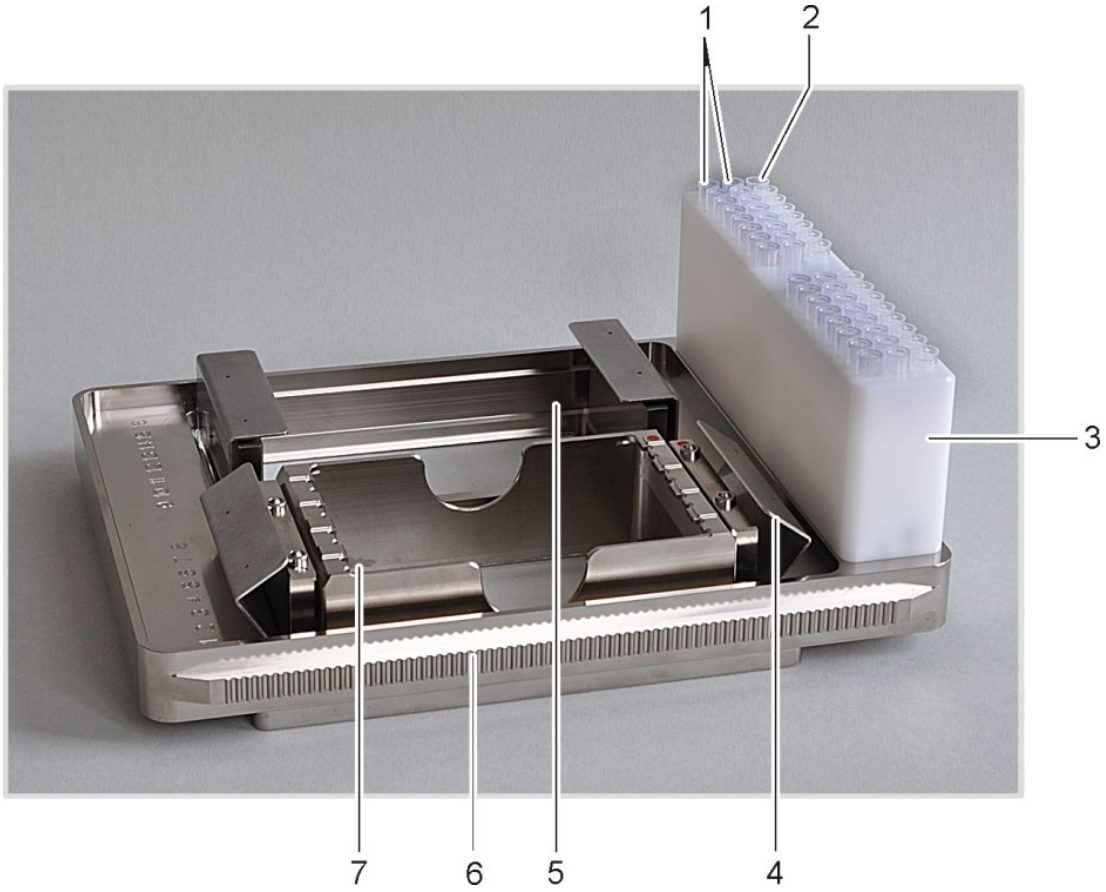
1. Transfer 50–100 mg stool sample into a 1.5 ml reaction tube.
2. Add **250 µl PBS** (not included in the kit). Vortex the tube for 10 seconds.
3. Centrifuge the tube at maximum speed for 3 minutes.
4. Transfer **50 µl of MAG Suspension** directly into the liquid of the **first cavity** of Reagent Plate.
5. Transfer **400 µl Lysis Solution V** into the **third cavity** of the Reagent Plate.
6. Add **200 µl PBS** to the **third cavity** of the Reagent Plate.
7. Add **200 µl** of the clear supernatant to the **third cavity** of the Reagent Plate.
8. Add **50 µl Proteinase K** to the **third cavity** of the Reagent Plate.
9. The sample will be processed using the InnuPure C16 touch. Please follow the instructions of chapter 13 p. 21.

12.5.2 Option 2: Fecal samples in ELISA buffer

1. Use **250** µl of the sample, transfer it into a 1.5 ml reaction tube and centrifuge the tube at maximum speed for 3 minutes.
2. Transfer **50 µl of MAG Suspension** directly into the liquid of the **first cavity** of Reagent Plate.
3. Transfer **400 µl Lysis Solution V** into the **third cavity** of the Reagent Plate.
4. Add **200 µl** of the clear supernatant to the **third cavity** containing Lysis Solution V.
5. Add **200 µl PBS** to the **third cavity** of the Reagent Plate.
6. Add **50 µl Proteinase K** to the **third cavity** of the Reagent Plate.
7. The sample will be processed using the InnuPure C16 touch. Please follow the instructions of chapter 13 p. 21.

13 Automated extraction using InnuPure C16 touch

13.1 Sample tray of InnuPure C16 touch



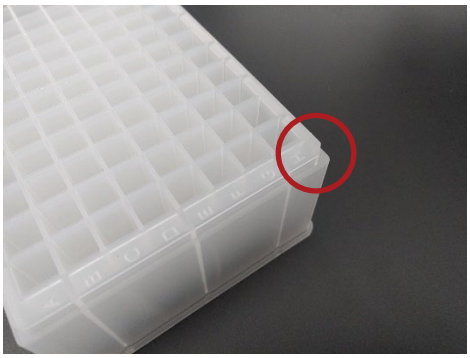
No. 1:	Filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Holding-down clamp
No. 5:	Sample block for Reagent Plates or adapter for Reagent Strips
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for Reagent Strips

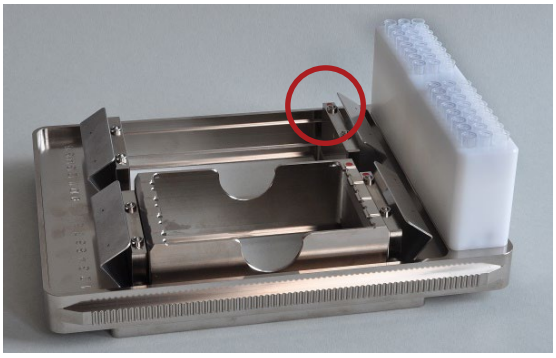
13.2 Preparing the sample tray of InnuPure C16 touch

1. Place the InnuPure 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 corner of the Reagent Plate must point to the colored dot on the holder.





CAUTION

Both holders have to be equipped with a Reagent Plate. If applicable, use an empty 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 hole at the edge of the tip block. Empty sample positions do not need to be filled.

NOTE

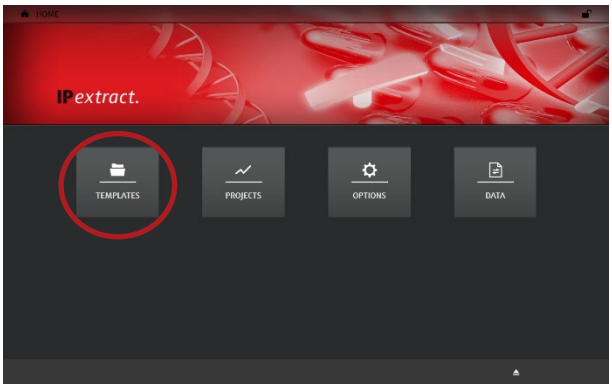
Make sure that for every sample the tips and the elution vessel are in the corresponding positions of the tip block!

IMPORTANT NOTE
It is possible to select between two different elution vessels! For small elution volumes up to 200 µl use Elution Strips (0.2 ml). For high elution volumes up to 500 µl use Elution Tubes (0.65 ml) with corresponding Elution Caps (Stripes).

13.3 Starting the InnuPure C16 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*.

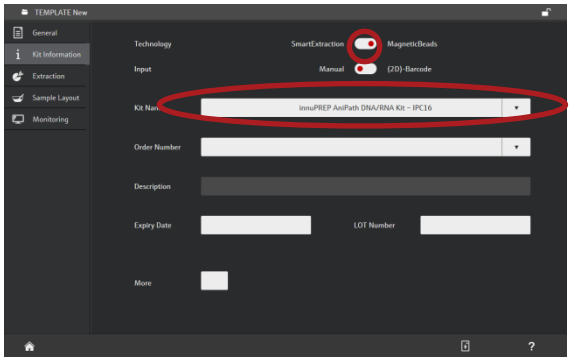
1. Switch on the InnuPure C16 *touch* and the tablet computer. Wait until the home screen of IP*extract* is displayed on the tablet screen.



NOTE
Home screen of IP*extract*

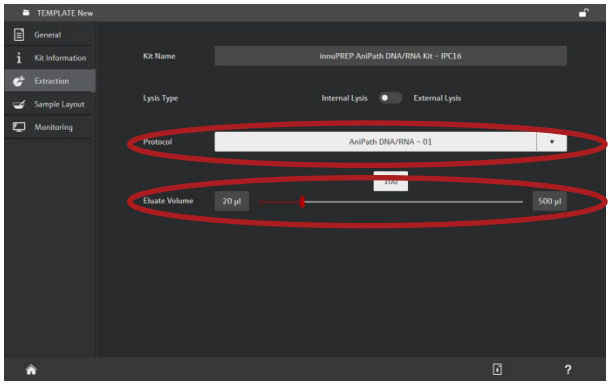
2. Choose [TEMPLATES]→[New Template]→[Kit-based].
3. Enter optional information in the tab “General”.
4. Choose the tab “Kit Information” and switch the “Technology” to “MagneticBeads”.

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



NOTE
“Kit Information” tab

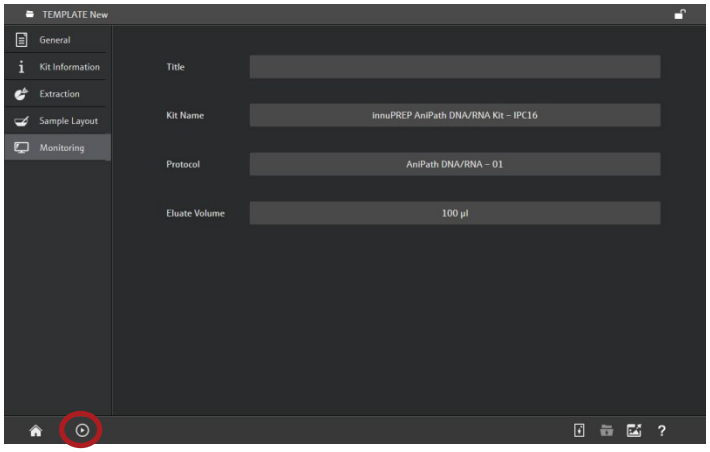
6. Enter optional information in the tab “Kit Information”
7. Choose the tab “Extraction” and choose the desired “Protocol”
   → “AniPath DNA/RNA - 01”
8. Adjust the “Eluate Volume“ using the slider or the text field.



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 or RNA 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
Poor lysis of starting material	
Insufficient disruption or homogenization	After lysis, centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no total RNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
Total RNA degraded	
RNA source inappropriately handled or stored	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, has been performed quickly.
RNase contamination of solutions; Receiver Tubes, etc.	Use sterile, RNase-free filter tips. Before every preparation clean the pipette, the devices and the work space. Always wear gloves!
Total RNA does not perform well in downstream applications (e.g. RT-PCR)	
Salt carryover during elution	Ensure that **Washing Solution A** and **Washing Solution B2** are at room temperature. Check Washing Solutions for salt precipitates. If there are any precipitates, dissolve them by careful warming.

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