Instructions for Use
Life Science Kits & Assays

innuPREP AniPath DNA/RNA Kit - PP Maxi

Innuscreen GmbH

Order No.:

845-PL-0030096	96 reactions
845-PL-0030960	960 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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Contents

1 Introduction

1.1 Intended use

The innuPREP AniPath DNA/RNA Kit - PP Maxi has been designed for automated isolation of viral or bacterial DNA and RNA from different kinds of starting material on the PurePrep Maxi. The extraction procedure is based on a new kind of chemistry.

The extraction procedure takes place on the magnetic particle processor PurePrep Maxi and allows the parallel and flexible extraction of 1 to 96 samples. The kit contains a Carrier Mix. The Carrier Mix consists of an essential Carrier RNA as well as a synthetic DNA fragment and MS2 RNA. Both can be used as internal extraction control which can be detected by using the innuDETECT Internal Control DNA/RNA Assay from IST Innuscreen GmbH. In addition, other internal controls can be used. No data are available on the rate of recovery of other internal controls. There can be no guarantee for the recovery of other internal controls. Other controls based on MS2 RNA sequences may lead to higher detection signals with the MS2 RNA from the Carrier Mix. It is important to note that the kit should always be used with an internal extraction control and corresponding detection assays to monitor the purification, amplification, and detection processes. Please note that the eluates contain Carrier Mix which might influence data from spectroscopic measurements. The detection limit for certain viruses depends on the individual procedures, for example in-house PCR or commercially used detection assays. We can give no warranty for the efficiency of extraction for different kinds of viruses.

The kit contains 2 different extraction protocols.
Protocol 1: Lysis and Binding steps are separated.
Protocol 2: Lysis and Binding steps are combined.
Protocol 1 was designed for maximum sensitivity and protocol 2 contains an uninterrupted run for maximum convenience.

The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions and further analytical procedures. Diagnostic results generated using the extraction procedure

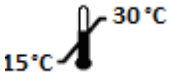
in conjunction with diagnostic tests should be interpreted regarding other clinical or laboratory results. To reduce irregularities in diagnostic results, internal controls for downstream applications should be used.

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> reactions.
	**Storage conditions** Store at room temperature or shown conditions respectively.
	**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 kit 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 shall only 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 could 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 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 into the RNA isolation.

4 Storage conditions

All kit components are shipped at ambient temperature.

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

Store lyophilized and dissolved **Carrier Mix** at -30 °C to -15 °C.

The mixture of **Lysis Solution V** and **Carrier Mix** is stable for a maximum of 7 days if stored at 2 °C to 8 °C.

All other components of the kit 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.

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

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 improve their performance and design. If you have any questions or problems regarding any aspects of the **innuPREP AniPath DNA/RNA Kit – PP Maxi** or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. For other countries please contact your local distributor.

6 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those referred to in the manual (→ "Product specifications" p. 10). 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 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 equivalents in other countries.

All products sold by 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

This kit is for research use only!

7 Kit components

7.1 Components included in the kit

	Σ 96	Σ 960
REF	845-PL-0030096	845-PL-0030960
Lysis Solution V	30 ml	250 ml
Carrier Mix	2 x for 1.25 ml working solution	12 x for 1.25 ml working solution
MAG Suspension F	1.1 ml	10 x 1.1 ml
Binding Solution V	60 ml	650 ml
Proteinase K	2 x for 1.5 ml working solution	16 x for 1.5 ml working solution
Washing Solution A	180 ml	2 x 850 ml
Washing Solution B2 (conc.)	48 ml	340 ml
Washing Solution ER	85 ml	2 x 450 ml
RNase free Water	15 ml	200 ml
Manual	1	1

7.2 Components not included in the kit

- 1.5 ml tubes
- 96 %–99.8 % ethanol (molecular biology grade, undenatured)
- ddH_2O; ultrapure for dissolving Proteinase K
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- 96 FlatWell plates, 96 DeepWell plate and tip combs for PP Maxi device (innuPREP Plate Set – PP Maxi (845-PLP-2000960), IST Innuscreen GmbH)

8 Product specifications

1. Starting material:

- Cell-free body fluids and cell culture supernatant (e.g. serum, plasma, cerebrospinal fluid)
- Whole blood samples (200 µl)
- Swabs from nasopharyngeal samples (e.g. Influenza testing or Covid testing)
- Tissue samples (up to 10 mg)
- Stool samples (50–100 mg)

2. Time for automated extraction protocol on PurePrep Maxi:

   Approx. 35 minutes

9 Usage of Carrier Mix

Besides 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. 11).

10 Initial steps before starting

- Add 1.5 ml ddH_2O to each vial of **Proteinase K**, mix thoroughly and store as described above.
- Add 1.25 ml RNase-free water (included) to each vial of **Carrier Mix**, mix thoroughly and store as described above.
- Add the indicated volume of absolute ethanol to **Washing Solution B2 (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-PL-0030096	Add 72 ml ethanol to 48 ml **Washing Solution B2**
845-PL-0030960	Add 510 ml ethanol to 320 ml **Washing Solution B2**.

- Prepare **Lysis Solution V / Carrier Mix**

Component	8 samples	48 samples	96 samples	n samples
Lysis Solution V	2 ml	12 ml	24 ml	250 µl x n samples
Carrier Mix	0.1 ml	0.6 ml	1.2 ml	12.5 µl x n samples
Final volume	2.1 ml	12.6 ml	25.2 ml	262.5 µl x n samples

NOTE

If the pre-mix is used directly, it is possible to add the Proteinase K according to the number of processed samples.

- Avoid freezing and thawing of starting material.

11 Sample Preparation

11.1 Protocol 1: Isolation from cell-free body fluids and cell culture supernatants

1. Label one Deep Well Plate “Lysis Plate”.
2. Transfer **200 µl** of the sample into the wells of the Deep Well Plate labeled “Lysis Plate”.
3. Add **200 µl Lysis Solution V /Carrier Mix** and add **20 µl Proteinase K** to each well used.
4. Proceed with “Extraction Protocol 1 ” on p.15.

11.2 Protocol 2: Isolation from 200 µl whole blood samples

1. Label one Deep Well Plate “Lysis Plate”.
2. Transfer **200 µl** of the whole blood sample into the wells of the Deep Well Plate labeled “Lysis Plate”.
3. Add **200 µl Lysis Solution V /Carrier Mix** and add **20 µl Proteinase K** to each well used.
4. Proceed with “Extraction Protocol 1 ” on p.15.

NOTE

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

11.3 Protocol 3: Isolation from nasopharyngeal samples (e.g. for Influenza testing)

1. Place the swabs into tubes containing physiological saline (0.9 % NaCl) or PBS and incubate under continuous shaking for 20 minutes.
2. Squeeze out the swab and remove the swab.

3. Label one Deep Well Plate “Lysis Plate”.
4. Transfer **200 µl** of the liquid sample into the wells of the Deep Well Plate labeled “Lysis Plate”.
5. Add **200 µl Lysis Solution V /Carrier Mix** and add **20 µl Proteinase K** to each well used.
6. Proceed with “Extraction Protocol 1 ” on p.15.

11.4 Protocol 4: Isolation from tissue homogenates

1. Transfer the tissue sample into a homogenization tube and add **400 µl ddH_20** (RNase-free) or **PBS**.
2. Homogenize the tissue samples using a commercially available bead-based homogenizer. For optimized results use 5-10 mg of tissue sample.
3. After homogenization centrifuge the sample at 10,000 x g for 2 minutes.
4. Label one Deep Well Plate “Lysis Plate”.
5. Transfer **200 µl** of the supernatant of the homogenized tissue sample into the wells of the Deep Well Plate labeled “Lysis Plate”.
6. Add **200 µl Lysis Solution V /Carrier Mix** and add **20 µl Proteinase K** to each well used.
7. Proceed with “Extraction Protocol 1 ” on p.15.

11.5 Protocol 5: Isolation from stool samples

NOTE
In some cases, the initial fecal sample is mixed with special ELISA Buffer for subsequent ELISA detection of different viruses. In this case use Option 2.

Option 1: Standard procedure

1. Transfer about 50–100 mg of 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. Label one Deep Well Plate “Lysis Plate”.
5. Transfer **200 µl** of the clear supernatant into the Deep Well Plate labeled “Lysis Plate”.
6. Add **200 µl Lysis Solution V / Carrier Mix** and add **20 µl Proteinase K** to each well used.
7. Proceed with “Extraction Protocol 1 ” on p.15.

Option 2: Fecal sample is already mixed with 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. Label one Deep Well Plate “Lysis Plate”.
3. Transfer **200 µl** of the clear supernatant into the Deep Well Plate labeled “Lysis Plate”.
4. Add **200 µl Lysis Solution V / Carrier Mix** and add **20 µl Proteinase K** to each well used.
5. Proceed with “Extraction Protocol 1 ” on p.15.

12 Extraction Protocol 1 (maximum sensitivity)

12.1 Prefilling of Plates

Label and fill the 96 Well plates according to the table below.

Plate	Position	Label	Content
Deep Well	1	Tip Comb Plate	96 Well Tip Comb
Deep Well	2	Lysis Plate	Lysed samples (including Lysis Solution V / Carrier Mix and Proteinase K)
Deep Well	3	Washing A	800 µl Washing Solution A
Deep Well	4	Washing A	800 µl Washing Solution A
Deep Well	5	Washing B2	800 µl Washing Solution B2
Deep Well	6	Washing ER	800 µl Washing Solution ER
Flat Well	8	Elution Plate	100 µl RNase-free Water

12.2 Loading the PurePrep Maxi with filled plates

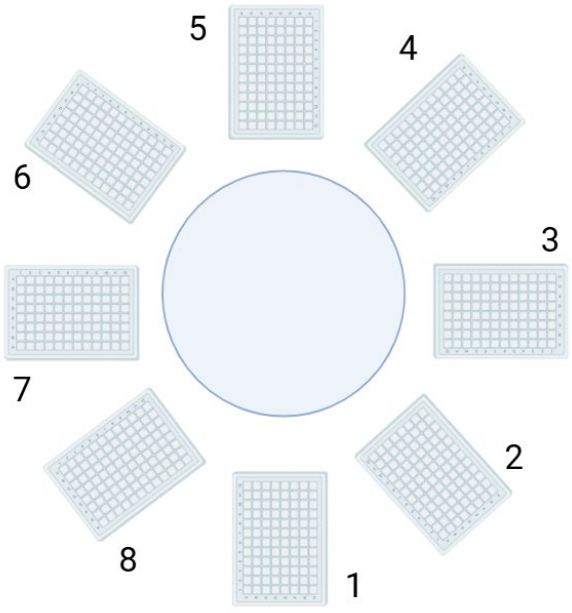


Fig. 1: Arrangement of the Plates in the device

1. Place the plates on the working table of the extraction device according to Fig.1.
2. Select the protocol “ANIPATH1“ and start the run.
3. The automated extraction process starts with sample lysis. After sample lysis the automated run stops.
4. After the device has stopped, take the Lysis Plate out of the device and add **10 µl** of well mixed **MAG Suspension F** and **560 µl** of **Binding Solution V** to the lysed samples.

NOTE

Mix the **MAG Suspension F** well by vortexing for 1 minute.

5. After adding **MAG Suspension F** and **Binding Solution V,** place the Lysis Plate back into the PurePrep Maxi and continue the extraction process by starting the device (you will find the instruction on the display of the PurePrep Maxi).
6. After finishing the extraction protocol, the Flat Well Plate contains the isolated DNA/RNA.

IMPORTANT NOTE

Store the DNA/RNA under adequate conditions.

We recommend storing the extracted RNA at −82 °C to −78 °C.

If the eluate contains carryover of magnetic particles, place the plate on a magnet or centrifuge the plate at maximum speed for 3 minutes. Pipet the supernatant with DNA/RNA into a new plate.

13 Extraction Protocol 2 (uninterrupted run)

13.1 Prefilling of Plates

Label and fill the 96 Well plates according to the table below.

NOTE
Filled plates can be sealed and stored at room temperature or 4°C for later use, if the run is performed on the same day

NOTE
Mix the **MAG Suspension F** well by vortexing for 1 minute.

Plate	Position	Label	Content
Deep Well	1	Tip Comb Plate	96 Well Tip Comb
Deep Well	2	Lysis Plate	Lysed samples (including Lysis Solution V / Carrier Mix and Proteinase K) **and** 560 µl Binding Solution V 10 µl well-mixed MAG Suspension F
Deep Well	3	Washing A	800 µl Washing Solution A
Deep Well	4	Washing A	800 µl Washing Solution A
Deep Well	5	Washing B2	800 µl Washing Solution B2
Deep Well	6	Washing ER	800 µl Washing Solution ER
Flat Well	8	Elution Plate	100 µl RNase-free Water

13.2 Loading the PurePrep Maxi with filled plates

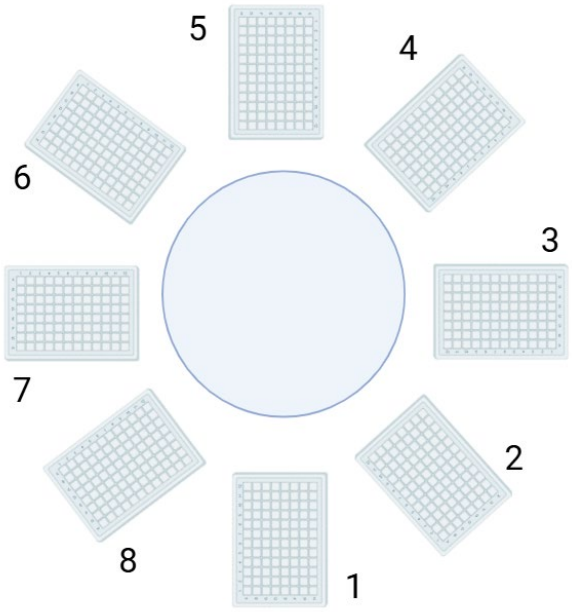


Fig. 1: Arrangement of the Plates in the device

1. Place the plates on the working table of the extraction device according to the Fig.1.
2. Select the protocol “ANIPATH2“ and start the run.
3. After finishing the extraction protocol, the Flat Well Plate contains the isolated DNA/RNA.

IMPORTANT NOTE

Store the DNA/RNA under adequate conditions.

We recommend storing the extracted RNA at −82 °C to −78 °C.

If the eluate contains carryover of magnetic particles, place the plate on a magnet or centrifuge the plate at maximum speed for 3 minutes. Pipet the supernatant with DNA/RNA into a new plate.

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.
No extracted DNA/ RNA	
Insufficient binding	Ensure that the MAG Suspension has been well mixed and added to the plates according to the instruction.
Low concentration of extracted DNA/RNA	
Eluate volume too high	Decrease the elution volume (min. 60 µl must be used!). The suggested elution volume is 100 µl. Please note that lowering the elution 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.
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 up the pipette, the devices and the working place. Always wear gloves!

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