

# Instructions for Use

## Life Science Kits & Assays



innuPREP AniPath DNA/RNA Kit - PP Mini, prefilled

**Order No.:**

845-PPA-1630016	16 reactions
845-PPA-1630096	96 reactions
845-PPB-1630016	16 reactions
845-PPB-1630096	96 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 - PP Mini, prefilled has been designed for automated isolation of viral or bacterial DNA and RNA from different kinds of starting material on the PurePrep Mini device. The extraction procedure is based on a new kind of chemistry.

The Reagent Plate / Strip of the kit is prefilled with almost all extraction reagents which are needed for the extraction process.

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 using the innuDETECT Internal Control DNA/RNA Assay by IST Innuscreen GmbH. In addition, user-specific internal controls can be used. No data are available on the rate of recovery of user-specific internal controls. There can be no guarantee for the recovery of user-specific internal controls. It is also pointed out here that user-specific 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. The detection limit for certain viruses depends on the individual procedures, for example in-house PCR or commercially used detection assays.

The kit contains 2 different extraction protocols.

Protocol 1: Lysis and Binding steps are separate

Protocol 2: Lysis and Binding steps are combined

Protocol 1 has superior sensitivity and protocol 2 is extremely fast and fully automated.

The extraction procedure takes place on the magnetic particle processor PurePrep Mini and allows the parallel and flexible extraction of 1 up to 16 samples. Additionally, the kit is compatible with the PurePrep Midi device. Please refer to the corresponding chapters of the devices.

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.

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### **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.

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## 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
	<b>REF</b> Catalogue number.
	<b>Content</b> Contains sufficient reagents for <N> reactions.
	<b>Storage conditions</b> Store at room temperature or shown conditions respectively.
	<b>Consult instructions for use</b> This information must be observed to avoid improper use of the kit and the kit components.
	<b>Expiry date</b>
	<b>Lot number</b> The number of the kit charge.
	<b>Manufactured by</b> Contact information of manufacturer.
	<b>For single use only</b> Do not use components for a second time.
	<b>Note / Attention</b> 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. 5).
- Working steps are numbered.

## 2 Safety precautions

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### 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.

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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.

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### FOR SINGLE USE ONLY!

This kit is made for single use only!

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### ATTENTION!

Don't eat or drink components of the kit!

The kit shall only be handled by educated personnel in a laboratory environment!

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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 potential 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.

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### ATTENTION!

Do not add bleach or acidic components to the waste after sample preparation!

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## 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.

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For more information on GHS classification and the Safety Data Sheet (SDS) please contact [sds.innu@ist-ag.com](mailto: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 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 **Carrier Mix** at -22 °C to -18 °C. Divide dissolved **Carrier Mix** into aliquots and store at -22 °C to -18 °C. Do not freeze and thaw the **Carrier Mix** more than 3 times.

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 solve these precipitates by careful warming. Before every use make sure that all components have 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 enhance their performance and design. If you have any questions or problems regarding any aspects of the **innuPREP AniPath DNA/RNA Kit – PP Mini, prefilled** 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.com](mailto:info.innu@ist.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. 11). 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 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.

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### NOTE

This kit is for research use only!

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## 7 Kit components

### 7.1 Components included in the kit

	 16	 96
<b>REF</b>	845-PPA-1630016 <sup>a</sup> 845-PPB-1630016 <sup>b</sup>	845-PPA-1630096 <sup>a</sup> 845-PPB-1630096 <sup>b</sup>
Lysis Solution V	6 ml	30 ml
Carrier Mix	1 x for 1.25 ml working solution	2 x for 1.25 ml working solution
MAG Suspension F	0.25 ml	1.1 ml
Binding Solution V	10 ml	60 ml
Proteinase K	2 x for 0.3 ml working solution	2 x for 1.5 ml working solution
Reagent Plate C (PP) <sup>a</sup>	1	6
Reagent Strip C (PP) <sup>b</sup>	16	96
Tip Combs <sup>a</sup>	2	12
Tip Combs <sup>b</sup>	4	24
Manual	1	1

### 7.2 Components not included in the kit

- 1.5 ml tubes
- ddH<sub>2</sub>O; ultrapure for dissolving Proteinase K
- RNase-free Water for dissolving Carrier Mix
- 96-well-plates or strips and tip combs for PurePrep Mini
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>)

## 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 Mini:

- Appr. 35 minutes

## 9 Initial steps before starting

- Add the indicated volume of ddH<sub>2</sub>O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-PPA-1630016	Add 0.3 ml ddH <sub>2</sub> O to lyophilized Proteinase K.
845-PPB-1630016	
845-PPA-1630096	Add 1.5 ml ddH <sub>2</sub> O to lyophilized Proteinase K.
845-PPB-1630096	

- Add the indicated volume of RNase-free Water to each vial of **Carrier Mix**, mix thoroughly and store as described above.

845-PPA-1630016	Add 1.25 ml RNase-free Water to lyophilized <b>Carrier Mix</b> .
845-PPB-1630016	
845-PPA-1630096	Add 1.25 ml RNase-free Water to lyophilized <b>Carrier Mix</b> .
845-PPB-1630096	

- Prepare **Lysis Solution V / Carrier Mix** according to the special protocols.

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

- Avoid freezing and thawing of starting material.

## 10 Preparing Reagent Plate / Strip for automated extraction

### 10.1 General filling scheme of the reagents (already prefilled)

Cavity of DW Plate/Strip	Content
Cavity 1	empty
Cavity 2	800 µl Washing Solution A
Cavity 3	800 µl Washing Solution A
Cavity 4	800 µl Washing Solution B2
Cavity 5	800 µl Washing Solution ER
Cavity 6	100 µl RNase-free Water

### 10.2 Unpacking of Reagent Plate and peeling off the sealing foil



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

Carefully open the overpack of Reagent Plates/Strips by using scissors.

Afterwards gently remove the foil by peeling off.

## 11 Sample Preparation

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

1. Transfer 200 µl of the sample into the first well of a Deep Well Plate/Strip.
2. Add 200 µl Lysis Solution V /Carrier Mix and add 20 µl Proteinase K to each well used.
3. Proceed with "Automated extraction using PurePrep Mini" on p.17.

### 11.2 Protocol 2: Isolation from 200 µl whole blood samples

1. Transfer 200 µl of the whole blood sample into the first well of the Deep Well Plate/Strip.
2. Add 200 µl Lysis Solution V /Carrier Mix and add 20 µl Proteinase K to each well used.
3. Proceed with "Automated extraction using PurePrep Mini" on p.17.

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#### NOTE

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

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### 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 continuously shaking for 20 minutes.
2. Squeeze out the swab and remove the swab.
3. Transfer 200 µl of the liquid sample into the first well of the Deep Well Plate/Strip.

4. Add 200 µl Lysis Solution V /Carrier Mix and add 20 µl Proteinase K to each well used.
5. Proceed with "Automated extraction using PurePrep Mini" on p.17.

#### 11.4 Protocol 4: Isolation from tissue homogenates

1. Homogenize the tissue samples using bead-based homogenizers. For optimized results use 5-10 mg of tissue sample.
2. Transfer the tissue sample into a homogenization tube and add 400 µl ddH<sub>2</sub>O (RNase free) or PBS.
3. After homogenization centrifuge the sample at 10,000 x g for 2 minutes.
4. Transfer 200 µl of the supernatant of the homogenized tissue sample into the first well of the Deep Well Plate/Strip.
5. Add 200 µl Lysis Solution V /Carrier Mix and add 20 µl Proteinase K to each well used.
6. Proceed with "Automated extraction using PurePrep Mini" on p.17.

#### 11.5 Protocol 5: Isolation from stool samples (e.g. from Norovirus)

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##### 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.

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##### 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. Transfer 200 µl of the clear supernatant into the first well of the Deep Well Plate/Strip.

## Sample Preparation

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5. Add **200 µl Lysis Solution V / Carrier Mix** and add **20 µl Proteinase K** to each well used.
6. Proceed with "Automated extraction using PurePrep Mini" on p.17.

### **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. Transfer **200 µl** of the clear supernatant into the first well of the Deep Well Plate/Strip.
3. Add **200 µl Lysis Solution V / Carrier Mix** and add **20 µl Proteinase K** to each well used.
4. Proceed with "Automated extraction using PurePrep Mini" on p.17.

## 12 Automated extraction using PurePrep Mini – Protocol 1

Addition of MAG Suspension F and Binding Solution V during the extraction is required.

### IMPORTANT

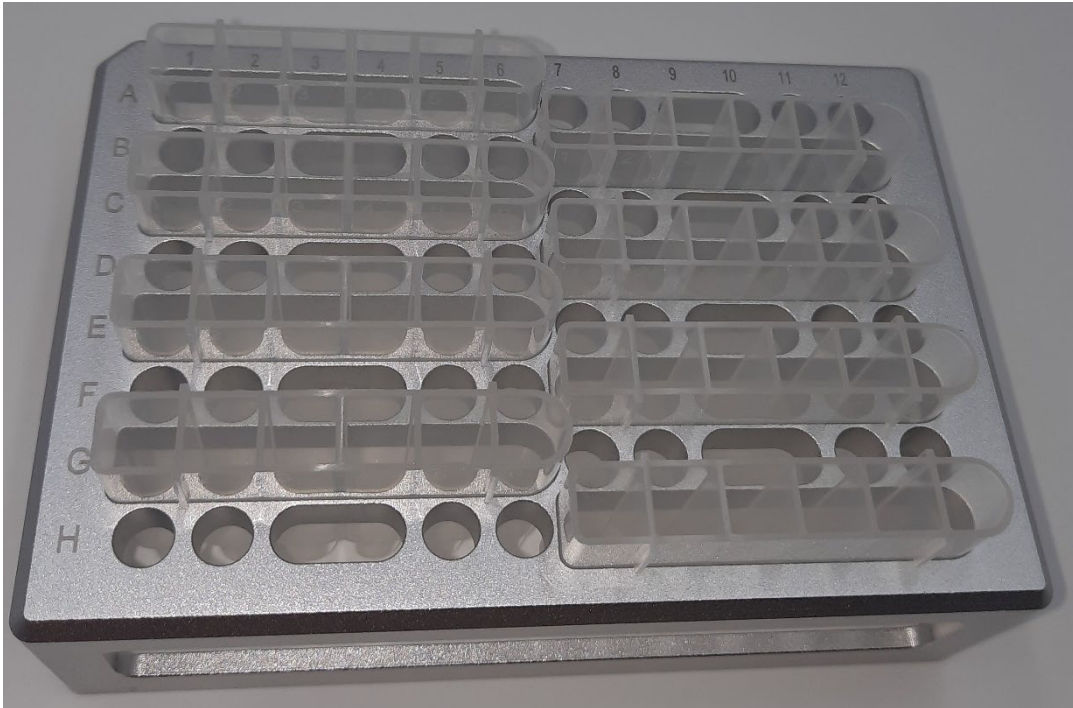
Make sure that all has been prepared as indicated (refer to p. 12 “Initial steps before starting”).

Vortex MAG Suspension F thoroughly before use!

### 12.1 Prefilled DW Plate or DW Strips

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	→				Eluate 1	Sample 9	→				Eluate 9
B	Sample 2	→				Eluate 2	Sample 10	→				Eluate 10
C	Sample 3	→				Eluate 3	Sample 11	→				Eluate 11
D	Sample 4	→				Eluate 4	Sample 12	→				Eluate 12
E	Sample 5	→				Eluate 5	Sample 13	→				Eluate 13
F	Sample 6	→				Eluate 6	Sample 14	→				Eluate 14
G	Sample 7	→				Eluate 7	Sample 15	→				Eluate 15
H	Sample 8	→				Eluate 8	Sample 16	→				Eluate 16

Fig. 1: Schematic illustration of DW Plate



**Fig. 2: Arrangement of the DW Strips in Tray**

### 12.2 Loading filled Deep Well Plate/Strips to the PurePrep Mini and plug in the Tip Combs

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#### NOTE

- When using strip (strips), the strip is inserted into the tray. In total, a maximum of 8 strips can be used in one extraction-run.
  - When working with strips, only every second tip is being used for extraction:  
Left tray side: Tip 1, 3, 5, 7  
Right tray side: Tip 2, 4, 6, 8.
  - It is recommended to mark the tips used for the extraction so that they are not used more than once
- 

1. Select the protocol  
"ANIPATH1" and start the run.

2. The automated extraction process starts with sample lysis. After sample lysis the automated run stops.
3. After the device has stopped, take the Plate/Strip 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 into **cavity 1**.

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**NOTE**

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

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4. After addition of **MAG Suspension F** and **Binding Solution V** place the Plate/Strip back to the PurePrep Mini and continue the extraction process by start the device (you will find the instruction on the display of the PurePrep Mini).
5. After finishing the extraction protocol, the Cavities 6 and 12 contain the isolated DNA/RNA.

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**IMPORTANT NOTE**

After finishing the extraction protocol, the last cavity of the Plate/Strip contains the isolated DNA/RNA. Pipet the supernatant with DNA/RNA into a new 1.5 ml elution tube. Store the DNA/RNA under adequate conditions.

We recommend storing the extracted DNA/RNA at  $-82\text{ }^{\circ}\text{C}$  to  $-78\text{ }^{\circ}\text{C}$ .

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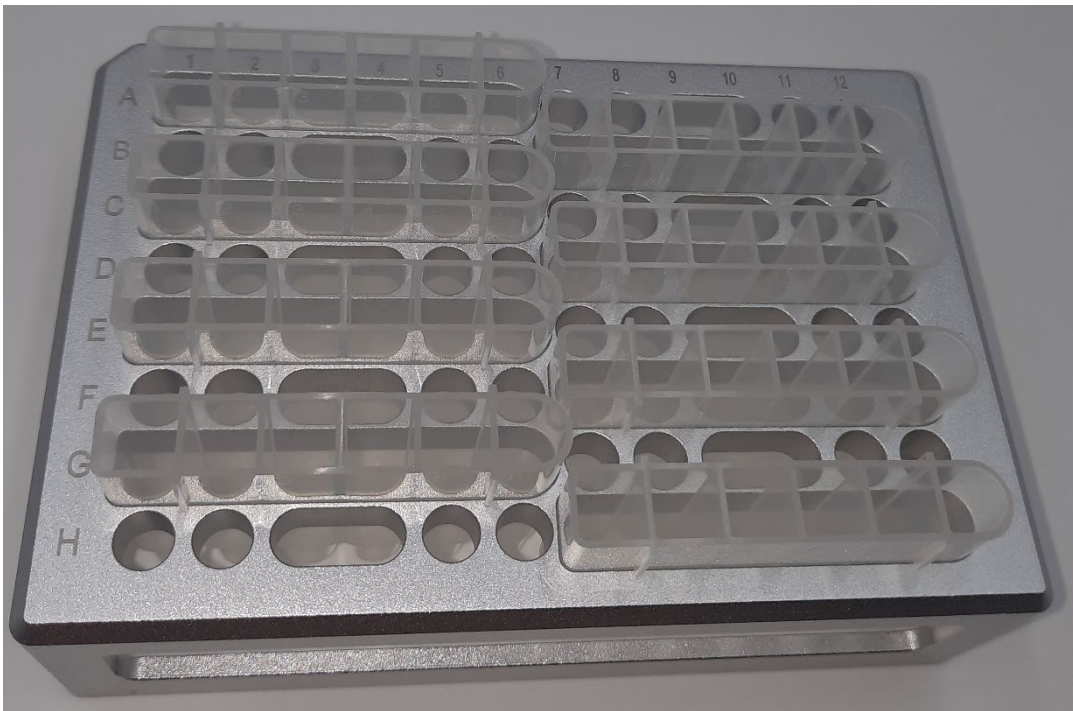
## 13 Automated extraction using PurePrep Mini – Protocol 2

Completely automated, no addition of MAG Suspension F and Binding Solution V during the run required. One run without stopping of the device.

### 13.1 Prefilled DW Plate or DW Strips

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	→				Eluate 1	Sample 9	→				Eluate 9
B	Sample 2	→				Eluate 2	Sample 10	→				Eluate 10
C	Sample 3	→				Eluate 3	Sample 11	→				Eluate 11
D	Sample 4	→				Eluate 4	Sample 12	→				Eluate 12
E	Sample 5	→				Eluate 5	Sample 13	→				Eluate 13
F	Sample 6	→				Eluate 6	Sample 14	→				Eluate 14
G	Sample 7	→				Eluate 7	Sample 15	→				Eluate 15
H	Sample 8	→				Eluate 8	Sample 16	→				Eluate 16

Fig. 1: Schematic illustration of DW Plate



**Fig. 2: Arrangement of the DW Strips in Tray**

Add to the prefilled plate:

Cavity of DW Plate/Strip	Content
Cavity 1 + 7	Sample + Lysis Solution V + 20 µl Proteinase K <b>and</b> 560 µl Binding Solution V 10 µl well mixed MAG Suspension F

### 13.2 Loading filled Deep Well Plate/Strips to the PurePrep Mini and plug in the Tip Combs

#### NOTE

- When using strip (strips), the strip is inserted into the tray. In total, a maximum of 8 strips can be used in one extraction-run.
- When working with strips, only every second tip is being used for extraction:

Left tray side: Tip 1, 3, 5, 7

Right tray side: Tip 2, 4, 6, 8.

- It is recommended to mark the tips used for the extraction so that they are not used more than once
- 

1. Select the protocol  
“ANIPATH2” and start the run.
  2. The automated extraction process starts with sample lysis.
  3. After finishing the extraction protocol, the Cavities 6 and 12 contain the isolated DNA/RNA.
- 

### **IMPORTANT NOTE**

After finishing the extraction protocol, the last cavity of the Plate/Strip contains the isolated DNA/RNA. Pipet the supernatant with DNA/RNA into a new 1.5 ml elution tube. Store the DNA/RNA under adequate conditions.

We recommend storing the extracted DNA/RNA at  $-82\text{ }^{\circ}\text{C}$  to  $-78\text{ }^{\circ}\text{C}$ .

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## 14 Troubleshooting

Problem / probable cause	Comments and suggestions
<b>Poor lysis of starting material</b>	
Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
<b>Little or no total RNA eluted</b>	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
<b>DNA contamination</b>	
Too much starting material	Reduce amount of starting material.
Incorrect lysis of starting material	Use the recommended techniques for lysis of cell pellet.
<b>Total RNA degraded</b>	
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!
<b>Magnetic beads carryover</b>	
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 or Elution vessels.

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