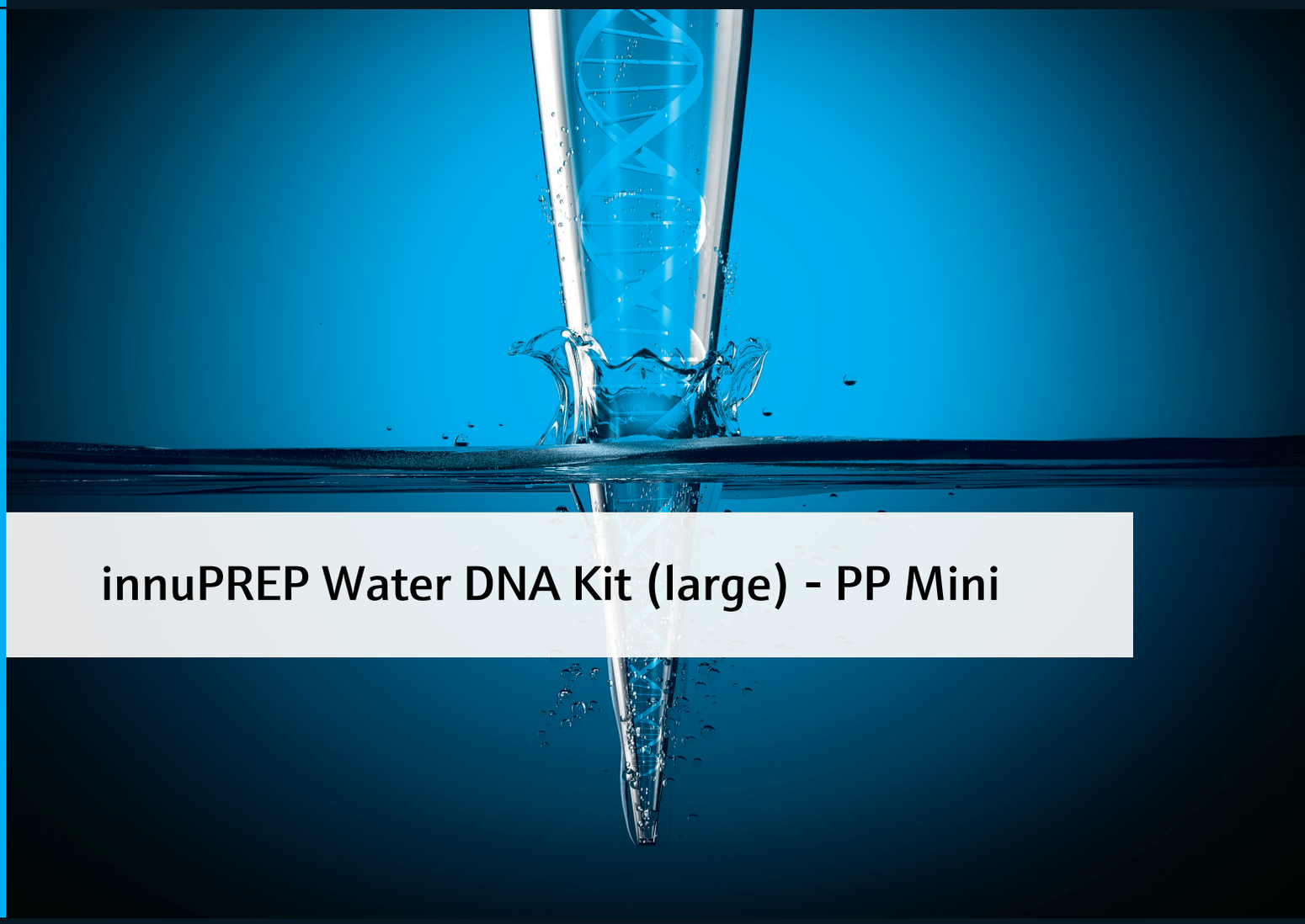
Instructions for Use
Life Science Kits & Assays



innuPREP Water DNA Kit (large) - PP Mini

Innuscreen GmbH

Order No.:

845-PS-0120016 16 reactions
845-PS-0120096 96 reactions

Publication No.: HB_PS-0120_e_250410

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Contents

1 Introduction

1.1 Intended use

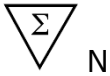
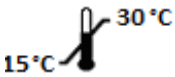
The **innuPREP Water DNA Kit (large) - PP Mini** has been developed for DNA extraction from large freshwater samples. This kit leverages two innovative technologies aimed at enhancing biomolecule enrichment. In the first step, the water sample is concentrated through a process known as Target Concentration Technology (TCT), which reduces the sample volume from 500 ml to 30-50 ml. No filtration step is necessary. In a second step, the technique Polymer Mediated Enrichment (PME) improves the capture and concentration of target biomolecules. PME selectively attracts and binds specific biomolecules such as viruses, bacteriophages, bacteria, environmental DNA (eDNA), algae, and other microorganisms or molecules of interest allowing them to pellet by a centrifugation step. The pellets are then processed using a PurePrep Mini device for DNA/ RNA extraction. The combination of these technologies enables the efficient extraction of genetic material from low or even trace concentrations of target biomolecules present in large water samples. The extracted DNA/RNA is of sufficient quality for a broad spectrum of downstream applications, including amplification reactions and various analytical procedures.

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 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. 3).
- 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 only 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 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 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival store **Enrichment Reagent VCR-1, MAG Suspension F** and **Proteinase K** at 4 – 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 have room temperature.

4 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 Kit 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.

5 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. 8). 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!

6 Kit components

6.1 Components included in the kit

	Σ 16	Σ 96
REF	845-PS-0120016	845-PS-0120096
TCT Beads (10 g)	16	96
Enrichment Reagent VCR-1	2 x 1.2 ml	7 x 1.2 ml
Enrichment Reagent VCR-2	2 x 2 ml	20 ml
Lysis Tube B 2.0	16	96
Lysis Solution RL	10 ml	50 ml
Proteinase K	2 x for 0.3 ml working solution	2 x for 1.5 ml working solution
Binding Solution V	10 ml	60 ml
MAG Suspension F	0.25 ml	1.1 ml
Washing Solution HS (conc.)	15 ml	90 ml
Washing Solution LS (conc.)	3 ml	20 ml
Washing Solution ER	17 ml	85 ml
RNase-free Water	2 x 2 ml	25 ml
Manual	1	1

6.2 Components not included in the kit

- 1000 ml bottles
- 50 ml centrifuge tubes
- 96–98.8 % ethanol (molecular biology grade, undenatured)
- DW Strip / DW Plate / DW Tip Comb (compatible with the PP Mini device) alternatively innuPREP Plate Set PP Mini (845-PSP-1000096) or innuPREP Strip Set PP Mini (845-PSS-2000096), IST Innuscreen GmbH

7 Initial steps before starting

- Add the indicated volume of absolute ethanol to **Washing Solution HS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-PS-0120016	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
845-PS-0120096	Add 90 ml ethanol to 90 ml Washing Solution HS (conc.).

- Add the indicated volume of absolute ethanol to **Washing Solution LS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-PS-0120016	Add 12 ml ethanol to 3 ml Washing Solution LS (conc.).
845-PS-0120096	Add 80 ml ethanol to 20 ml Washing Solution LS (conc.).

- Add the indicated volume of ddH_2O to each vial of **Proteinase K** and mix thoroughly. Store as described above.

845-PS-0120016	Add 0,3 ml ddH_2O to 6 mg Proteinase K.
845-PS-0120096	Add 1,5 ml ddH_2O to 30 mg Proteinase K.

8 Product specifications

1. Starting material:

- 500 ml water

2. Total time including automated extraction protocol on PurePrep Mini:

- Preparatory work: approx. 135 minutes
- Extraction: 45 minutes

9 Sample preparation

9.1 Volume reduction based on TCT-Technology (reduction to 50 – 30 ml)

1. Transfer 500 ml of water sample into a 1000 ml bottle.
2. Add the **TCT Beads (10g)** and shake the bottle.
3. Incubate the bottle at ambient temperature until the initial volume is reduced to 30 – 50 ml.

> **NOTE**
> Incubation time depends on water sample. Standard drinking water will reduce to 30 – 50 ml within 120 minutes; water containing fertilizer and other components need a longer incubation time.

4. Shake the bottle vigorously. Transfer the reduced water sample (30 – 50 ml) into a 50 ml tube.

9.2 Target enrichment based on PME-Technology (complexing of biomolecules – viruses, bacteria, fungi, bacteriophages, protozoa, plant and free DNA)

1. Add **75 µl Reagent VCR-1** and **150 µl Reagent VCR-2** into the water sample.
2. Mix shortly and incubate the tube at ambient temperature for 10 minutes.
3. Centrifuge the sample for 10 minutes at 4.000 x g.
4. Carefully remove the supernatant as much as possible.

> **NOTE**
> In some samples, the pellet may be very loose. Therefore, the supernatant should be removed very carefully, using a pipette if necessary.

5. Add **500 µl Lysis Solution RL** to the pellet and resuspend the pellet completely by pipetting up and down 10 times.

> **NOTE**
> It is important to pipette carefully up and down at least 10 times, as complexed biomolecules may be located at the bottom below the sediment pellet, which do not dissolve so quickly.

6. Transfer the dissolved pellet to Lysis Tube B 2.0, vortex shortly and start the homogenization step for 30s at 6.500 x g.

> **NOTE**
> The homogenization process using commercially available homogenizers (Precellys, Fastprep, Bead Raptor etc.) can be changed and optimized depending on the used homogenizer. The optimal duration and intensity of homogenization depends on which kind of homogenizer is used.

7. Centrifuge for 2 minutes at 11.000 x g.
8. Proceed with 10 “Automated extraction using PurePrep Mini”

10 Automated extraction using PurePrep Mini

10.1 Prefilling of the DW Plate or the DW Strips

1. Carefully transfer **the supernatant from bead beating (max. 400 µl)** into **the first cavity** of the DW Strip or the DW Plate.

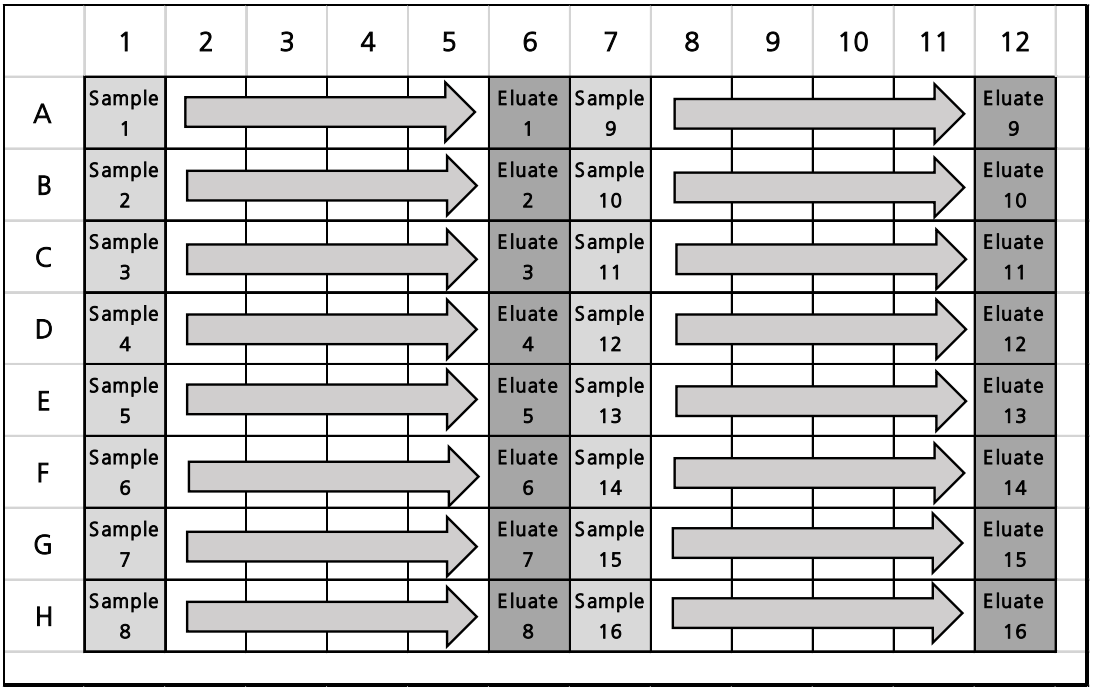


Fig. 1: Schematic illustration of DW Plate

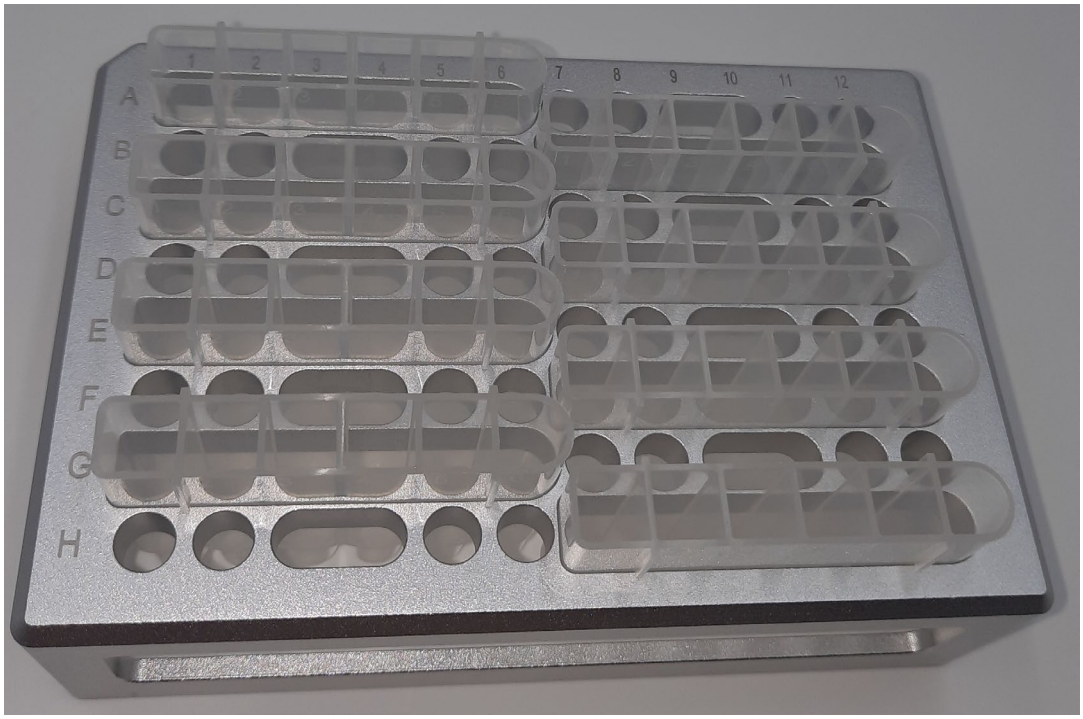


Fig. 2: Arrangement of the DW Strips in Tray

Cavity of DW Plate/Strip	Content
Cavity 1 + 7	400 µl supernatant + 20 µl Proteinase K + 10 µl MAG Suspension F + 550 µl Binding Solution V
Cavity 2 + 8	800 µl Washing Solution HS
Cavity 3 + 9	800 µl Washing Solution HS
Cavity 4 + 10	800 µl Washing Solution LS
Cavity 5 + 11	800 µl Washing Solution ER
Cavity 6 + 12	100 – 200 µl RNase-free Water

The prefilling is carried out from left to right as shown in the illustration, Fig. 1. The DW Strips located in the tray are filled in the same way.

10.2 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 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 “Water1“ and start the run.
2. The automated extraction process starts with sample lysis. After sample lysis the automated run stops.
3. Place the Plate/Strip back to the PurePrep Mini and continue the extraction process by confirming the button on the display of the device.
4. After finishing the extraction protocol, the cavities 6 and 12 contain the isolated DNA/RNA.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA/RNA despite a large pellet as a starting material	
Insufficient lysis	Prolong homogenization time up to 2 minutes.
Low concentration of extracted DNA/RNA	
Too much RNase-free Water	Elute the DNA/RNA in a lower volume of RNase-free Water (min. 80 µl).

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