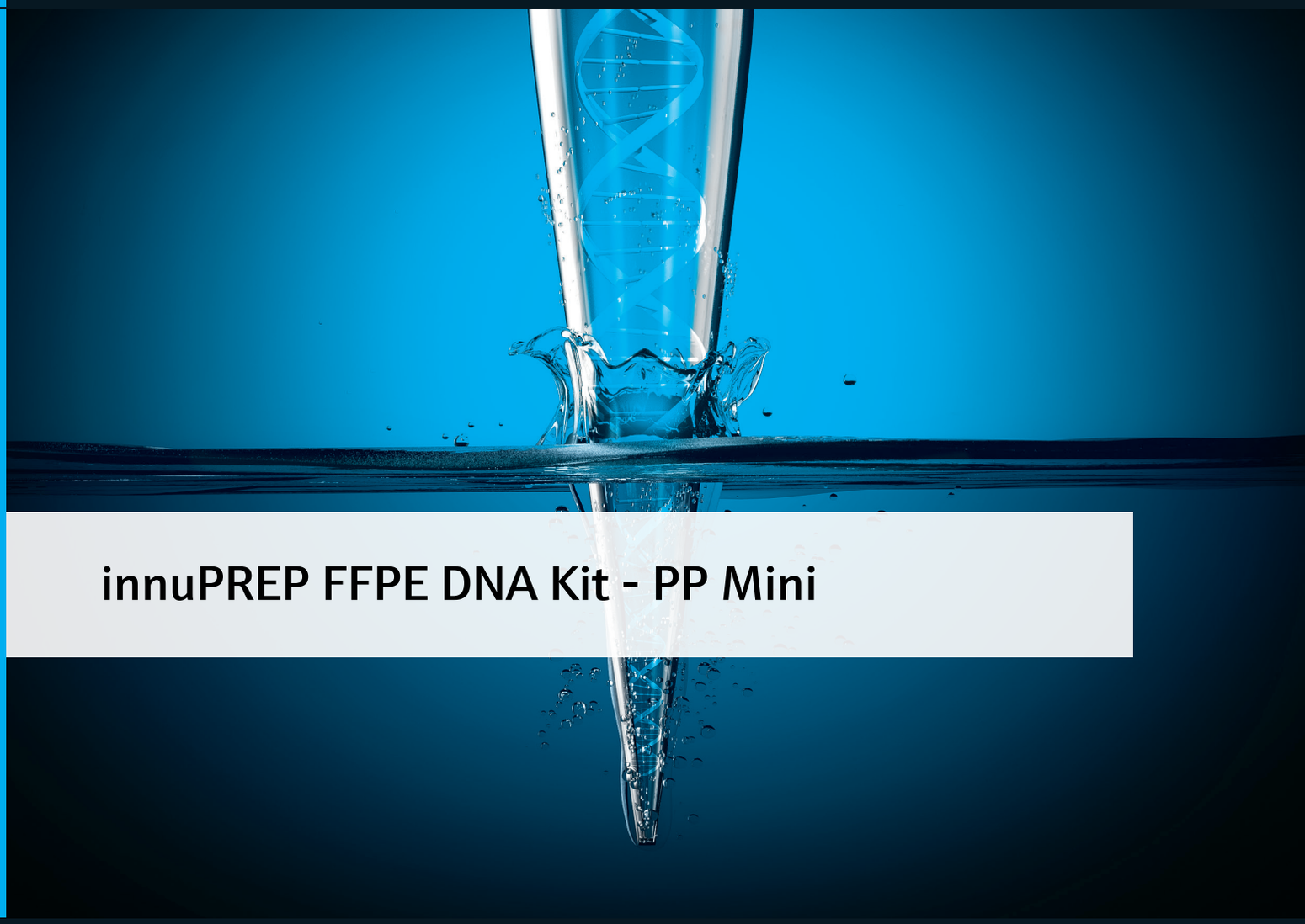
Instructions for Use
Life Science Kits & Assays



innuPREP FFPE DNA Kit - PP Mini

Innuscreen GmbH

Order No.:

845-PS-0040016	16 reactions
845-PS-0040096	96 reactions

Publication No.: HB_PS-0040_e_250827

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Contents

1 Introduction

1.1 Intended use

The **innuPREP FFPE DNA Kit – PP Mini** has been designed for automated isolation of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) samples using the PurePrep Mini device. The extraction procedure is based on a new patented chemistry.

The procedure starts with an external lysis step without the need for a deparaffinization step using toxic and hazardous components like octane or xylene. After the external lysis and incubation step the following extraction process runs automatically on the PurePrep Mini. The extraction process is based on binding of the DNA to surface-modified magnetic particles. After washing steps the nucleic acid is eluted from the magnetic particles with Elution Buffer and is now ready to use.

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.

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.

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	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15 °C 30 °C	**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	**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. 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 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 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.

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

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.

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.

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 **innuPREP FFPE DNA Kit – PP Mini** 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. 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 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!

6 Kit components

6.1 Components included in the kit

	Σ 16	Σ 96
REF	845-PS-0040016	845-PS-0040096
Lysis Solution BC	4 ml	20 ml
Solution QPS	4 ml	25 ml
MAG Suspension F	0.25 ml	1.1 ml
Binding Solution SBS	8 ml	45 ml
Proteinase K	1 x for 1,5 ml working solution	3 x for 1,5 ml working solution
Washing Solution C	15 ml	80 ml
Washing Solution LS (conc.)	6 ml	40 ml
RNase-free Water	6 ml	25 ml
Manual	1	1

6.2 Components not included in the kit

- 2.0 ml tubes
- 96 %–99.8 % ethanol (molecular biology grade, undenatured)
- ddH_2O; ultrapure for dissolving Proteinase K
- DW Strip / DW Plate / DW Tip Comb (compatible with the PP Mini device)

7 Product specifications

1. Starting material:
   - FFPE tissue samples
   - Size: approx. 2 × 5 µm; optionally more starting material

2. Time for isolation:
   Preliminary steps: approx. 3.5 hours
   Automated Extraction protocol: approx. 30 minutes

8 Initial steps before starting

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

845-PS-0040016	Add 1.5 ml ddH_2O to lyophilized **Proteinase K**.
845-PS-0040096	Add 1.5 ml ddH_2O to lyophilized **Proteinase K**.

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

845-PS-0040016	Add 24 ml ethanol to 6 ml **Washing Solution LS (conc.)**.
845-PS-0040096	Add 160 ml ethanol to 40 ml **Washing Solution LS (conc.)**.

9 Sample Preparation

9.1 Lysis of FFPE tissue samples

NOTE
The lysis of the starting material is a preliminary manual step. Heat two thermomixers or water baths to 65 °C and 90 °C, respectively.

1. Place the FFPE tissue sample (approx. 2x 5 µm or 1x 10 µm; optional more starting material) into a 2.0 ml reaction tube, close the cap and centrifuge the reaction tube at max. speed for 1 minute.
2. Open the reaction tube and add **200 µl Lysis Solution BC** and **40 µl Proteinase K** to the sample and mix vigorously by pulsed vortexing for 10 seconds. Centrifuge briefly to remove drops from the lid of the tube.

NOTE
The FFPE tissue sample must be completely covered by Lysis Solution BC!

3. Incubate the reaction tube at 65 °C for 2.5 hours in a thermal mixer under continuous shaking at 1.000 rpm.

NOTE
Overnight incubation at 65 °C may increase the DNA yield. However, for most types of FFPE tissue sample the incubation at 65 °C for 2.5 hours is sufficient. We recommend using a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation.

4. After the lysis step place the sample into a second thermal mixer pre-heated to 90 °C and incubate the sample for 1 hour.

NOTE
Do not place the sample into the thermal mixer, before the temperature of 90 °C is achieved!

5. Open the reaction tube and add **200 µl Solution QPS** to the sample, mix vigorously by pulsed vortexing for 5 seconds.

6. Centrifuge the reaction tube at max. speed for 3 minutes.

NOTE

If there is a thin film of paraffin above the sample, pierce this film carefully with pipette and carefully remove the sample.

9.2 Protocol: Isolation from FFPE tissue samples

1. Transfer **400 µl** of the lysed sample to the Deep Well Plate/Strip according to the illustration (Fig. 1).

2. Afterwards add **400 µl Binding Solution V** and **10 µl MAG Suspension F** to each well used.

3. Proceed with “Automated extraction using PurePrep Mini” on p. 11.

10 Automated extraction using PurePrep Mini

10.1 Prefilling of the DW Plate or the DW Strips

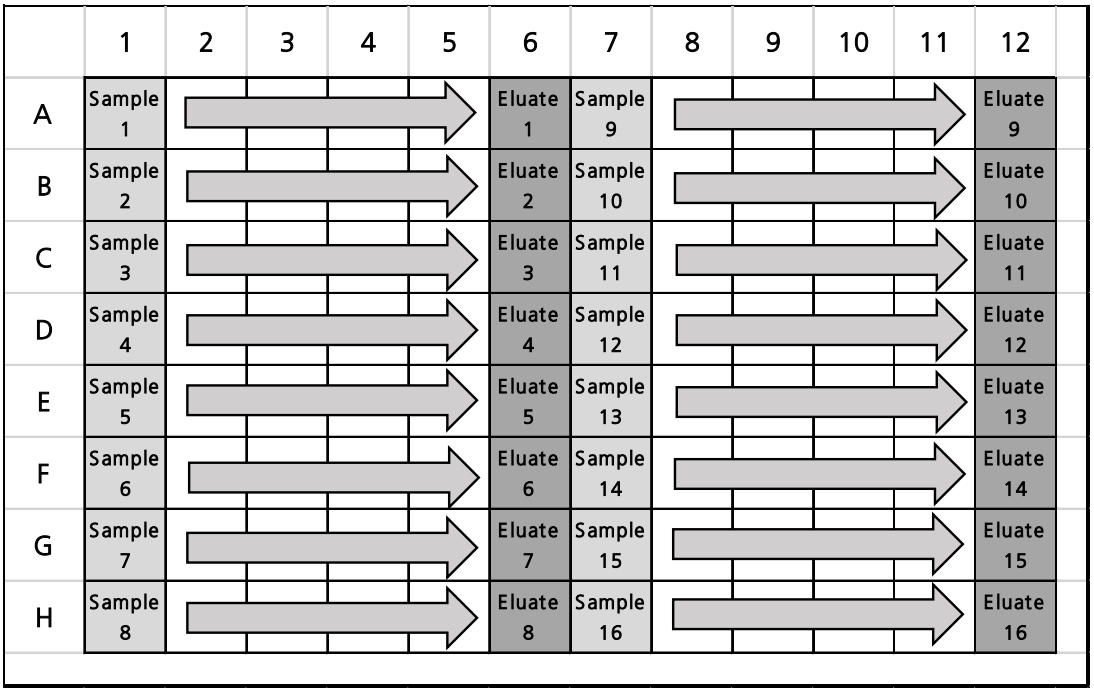

Fig. 1: Schematic illustration of DW Plate

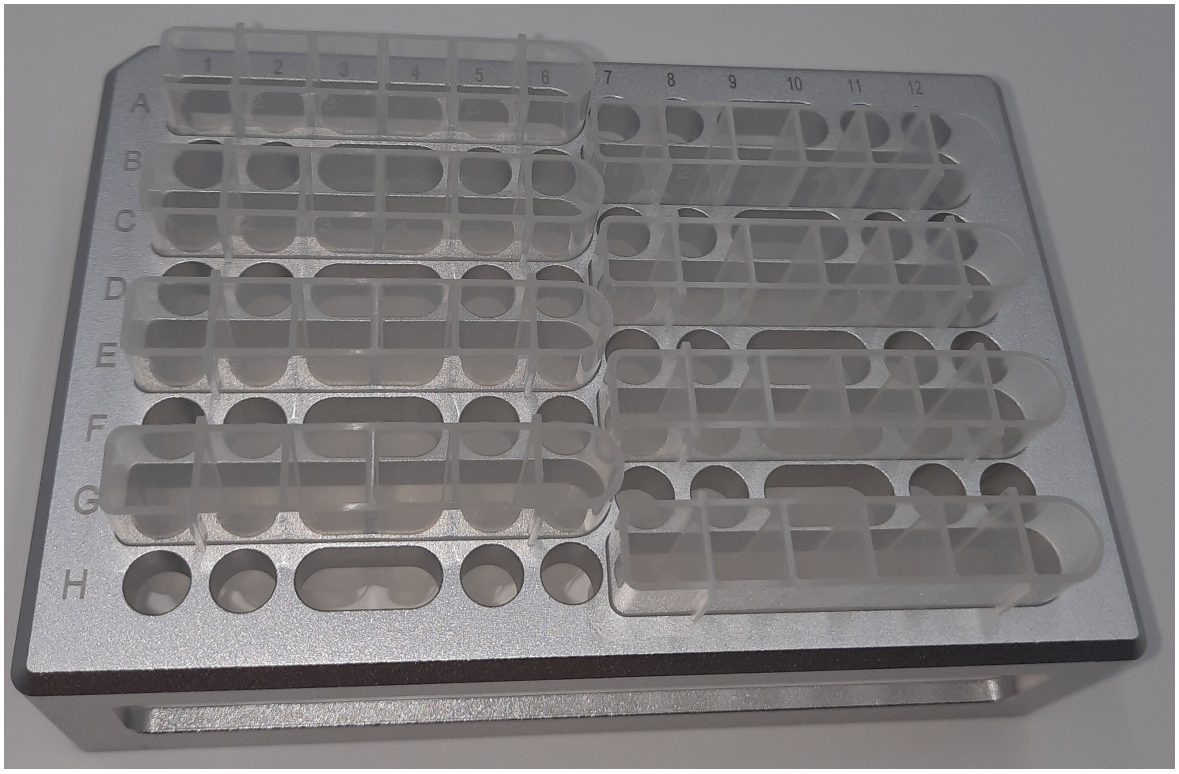

Fig. 2: Arrangement of the DW Strips in Tray

Cavity of DW Plate/Strip	Content
Cavity 1	400 µl lysed sample + 400 µl Binding Solution SBS + 10 µl MAG Solution F
Cavity 2	800 µl Washing Solution C
Cavity 3	800 µl Washing Solution LS
Cavity 4	800 µl Washing Solution LS
Cavity 5	800 µl Ethanol absolute
Cavity 6	150 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 strip (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

   “GDNA2“ and start the run.
2. After finishing the extraction protocol, the Cavities 6 and 12 contain the isolated DNA.
3. Transfer the DNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

After finishing the extraction protocol, the last cavity of the Plate/Strip contains the isolated DNA. Store the DNA under adequate conditions.

We recommend storing the extracted DNA for longer use at −22°C to -18 °C.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Prolong lysis time. Reduce amount of starting material.
Low concentration of extracted DNA	
Too much Elution Buffer (RNase-free Water)	Elute the DNA in a lower volume of RNase-free Water (min. 80 µl).
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 into a new plate or Elution vessels

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