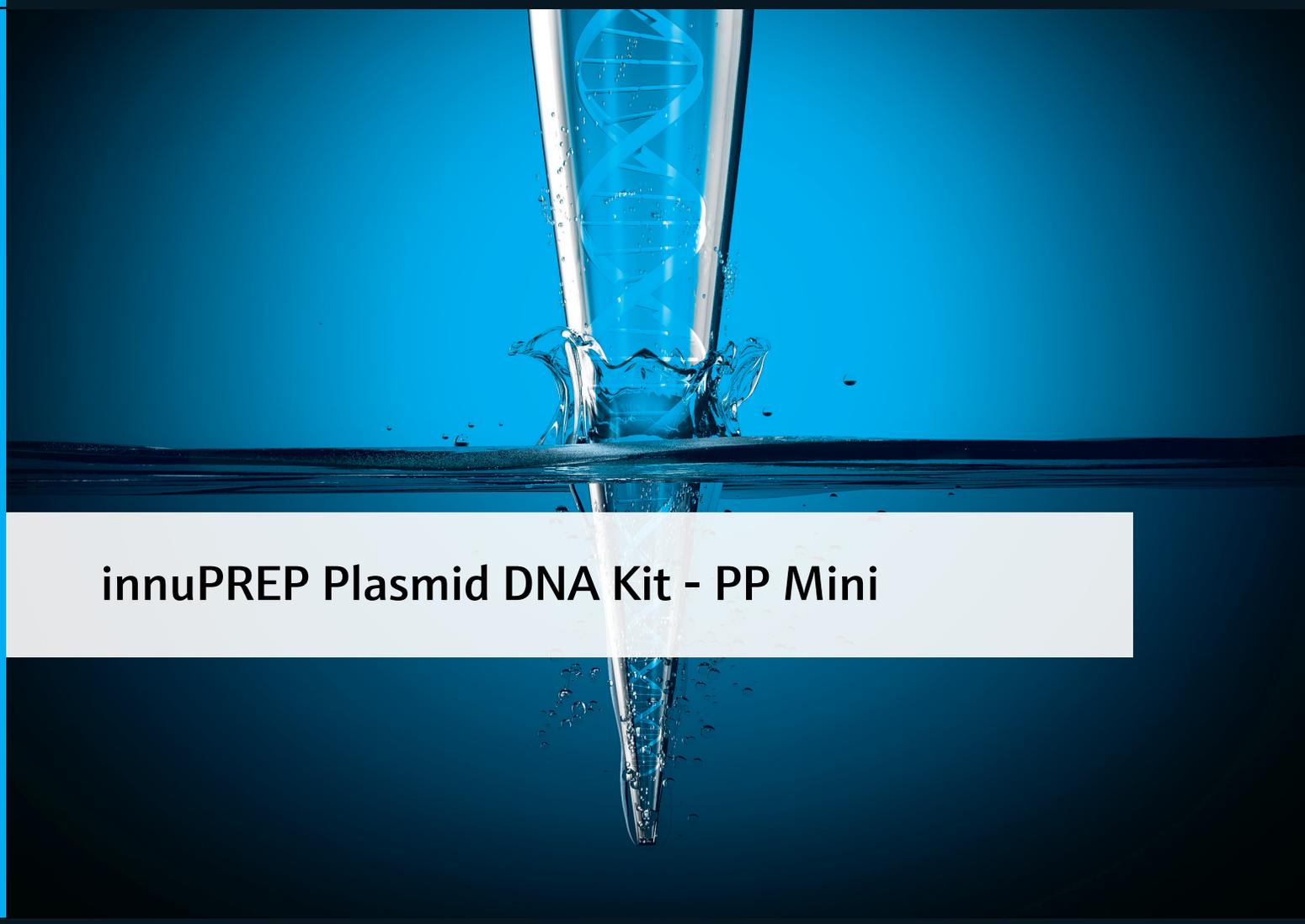
Instructions for Use
Life Science Kits & Assays



innuPREP Plasmid DNA Kit - PP Mini

Innuscreen GmbH

Order No.:

845-PS-0130016	16 reactions
845-PS-0130096	96 reactions

Publication No.: HB_PS-0130_e_250827

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Contents

1 Introduction

1.1 Intended use

The **innuPREP Plasmid DNA Kit – PP Mini** has been designed for the automated isolation of plasmid DNA from up to 5 ml of cultured bacterial cells on the PurePrep Mini device.

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 extraction process based on an alkaline lysis procedure combined with binding of plasmid DNA (pDNA) on the surface of the magnetic particle. After binding the pDNA is washed to remove RNA, proteins or other impurities. The eluted plasmid DNA is from excellent quality and therefore highly suited for several downstream applications like transfection, cloning, sequencing, PCR or in vitro transcription.

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“ 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 **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 Plasmid 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-0130016	845-PS-0130096
Resuspension Buffer	6 ml	30 ml
Lysis Buffer	6 ml	30 ml
Neutralization Buffer	8 ml	40 ml
MAG Suspension F	0.25 ml	2 x 1.1 ml
Washing Solution A	15 ml	90 ml
Washing Solution B (conc.)	8 ml	40 ml
RNase-free Water	15 ml	100 ml
Elution Buffer P	2 ml	15 ml
Manual	1	1

6.2 Components not included in the kit

- 1.5 ml or 2.0 ml reaction tubes or 15 ml reaction tubes (optional)
- 96 %–99.8 % ethanol (molecular biology grade, undenatured)
- DW Strip / DW Plate / DW Tip Comb (compatible with the PP Mini device)

7 Product specifications

1. Starting material:

- Bacterial culture (0.5-5.0 ml) for isolation of high copy plasmid DNA
- Bacterial culture (0.5-5.0 ml) for isolation of low copy plasmid DNA
- Bacterial culture (0.5-5.0 ml) for isolation of cosmid DNA

2. Time for automated extraction protocol on PurePrep Mini:

- Appr. 35 minutes

8 Initial steps before starting

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

845-PS-0130016	Add 12 ml ethanol to 8 ml **Washing Solution B**.
845-PS-0130096	Add 60 ml ethanol to 40 ml **Washing Solution B**.

9 Protocol 1: Isolation of plasmid DNA from 0.5 -5 ml bacterial culture

1. Transfer **0.5 ml up to 5 ml** of the overnight *E. coli* culture into a 1.5 ml, 2.0 ml or 15 ml reaction tube. Centrifuge for 1 minute at maximum speed to pellet the bacteria; remove the supernatant as completely as possible.
2. Resuspend the bacterial cell pellet in **250 µl Resuspension Buffer** completely by vortexing or by pipetting up and down.
3. Transfer **250 µl** of the resuspended bacterial pellet according to the illustration (Fig.1) to the Deep Well Plate/Strip.
4. Add **250 µl Lysis Buffer** to each used well and incubate for 1 minute.
5. Afterwards add **350 µl Neutralization Buffer** and **10 µl MAG Suspension F** to each well used.
6. Proceed with “Automated extraction using PurePrep Mini” on p.10.

ATTENTION

Please check **Lysis Buffer** for precipitates. If white precipitates are visible, warm the **Lysis Buffer** several minutes at 30 °C–40 °C until the precipitates are dissolved. Cool **Lysis Buffer** down to room temperature.

10 Automated extraction using PurePrep Mini

10.1 Prefilling of the DW Plate or the 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

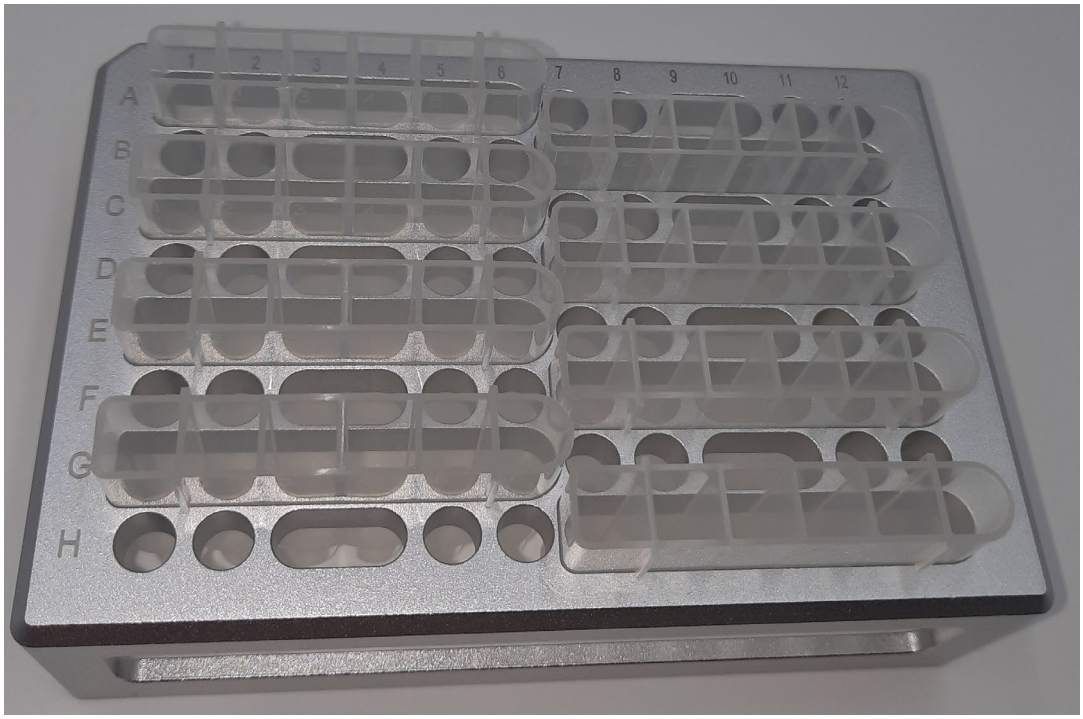

Fig. 2: Arrangement of the DW Strips in Tray

Cavity of KF96 DW Plate/Strip	Content
Cavity 1	Sample + 250 µl Lysis Buffer + 350 µl Neutralization Buffer + 10 µl MAG Suspension F
Cavity 2	800 µl Washing Solution A
Cavity 3	800 µl Washing Solution B
Cavity 4	200 µl Washing Solution B
Cavity 5	800 µl RNase-free Water
Cavity 6	100 µl Elution Buffer P

The prefilling is caried 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 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

   “Plasmid1” 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 **5 µl** of well mixed **MAG Suspension F** to the lysed samples in cavity 1.

NOTE

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

4. After addition of **MAG Suspension F** place the Plate/Strip back to the PurePrep Mini and continue the extraction process by starting 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 pDNA.

6. Transfer the pDNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

After finishing the extraction protocol. Store the extracted DNA at 4–8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low recovery	
Incorrect **Washing Solution B** or no ethanol added	Prepare the **Washing Solution B** exactly as described in the manual. Store the **Washing Solution B** with firmly fixed cap.
Low concentration of extracted DNA	
Too much Elution Buffer P	Elute the pDNA in a lower volume of Elution Buffer P (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 pDNA into a new plate or Elution vessels.

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