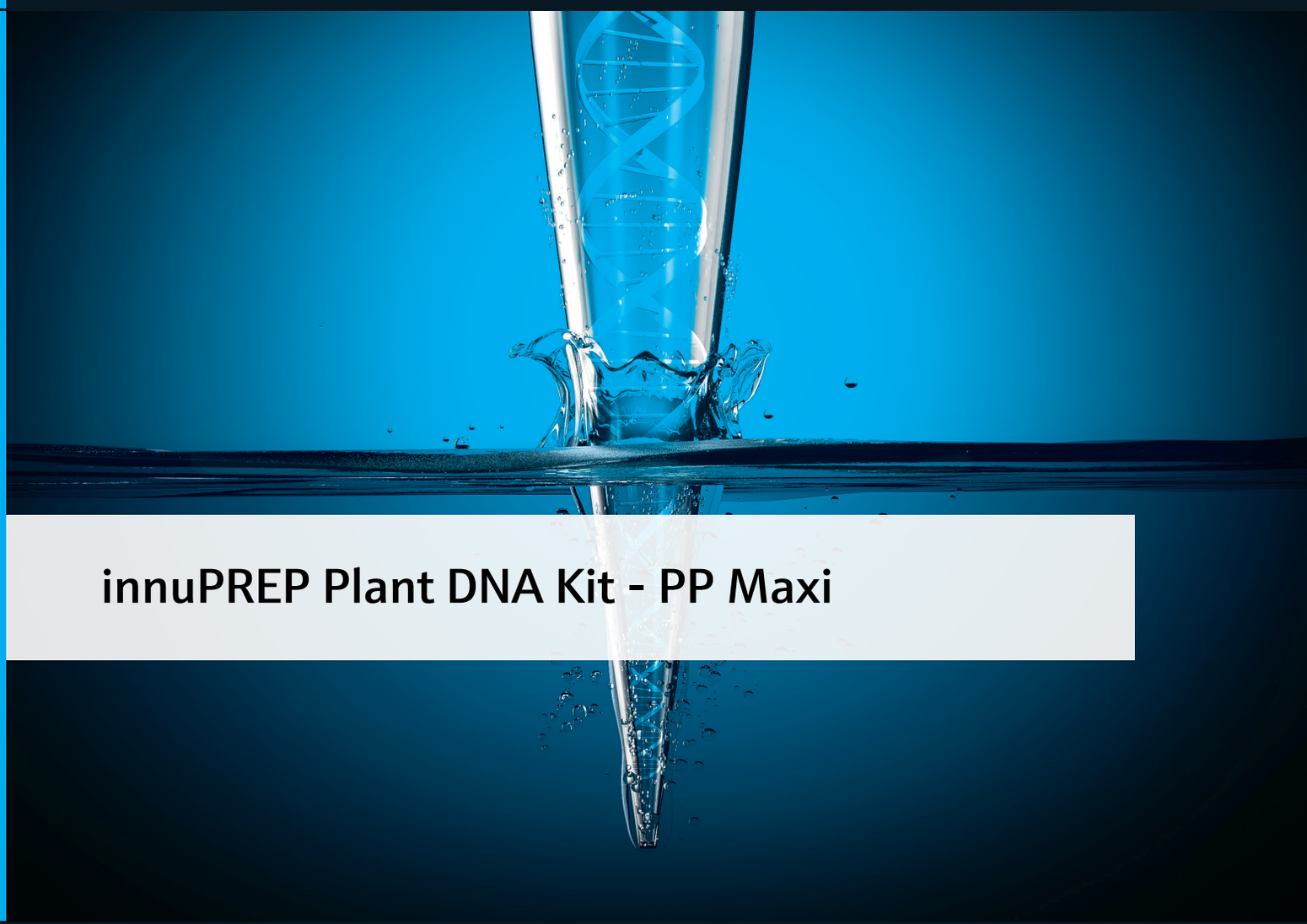
Instructions for Use
Life Science Kits & Assays



innuPREP Plant DNA Kit - PP Maxi

Innuscreen GmbH

Order No.:

845-PL-0050096	96 reactions
845-PL-0050960	960 reactions

Publication No.: HB_PL-0050_e_250331

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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1 Introduction

1.1 Intended use

The **innuPREP Plant DNA Kit – PP Maxi** has been designed for the automated isolation of genomic DNA from plant samples on the PurePrep Maxi device. 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 up to 96 samples.

The procedure starts with an external lysis step of homogenized plant material followed by the automated extraction of genomic DNA. The kit has been tested for isolation of genomic DNA from leaves, fruits, woods, needles as well as seeds. The starting material can be fresh or frozen. For optimal lysis of plant material, the kit contains two different Lysis Solutions. Following lysis, the samples are cleared by centrifugation or precipitation.

Afterwards, the samples are transferred into a DW Plate (available separately). The following extraction process runs automatically on the PurePrep Maxi and is based on the binding of DNA to the surface of the modified magnetic particles. After washing steps, the nucleic acid is eluted and is now ready-to-use for downstream applications.

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 might 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 Storage conditions

All kit components are shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K and MAG Suspension** 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 Plant DNA 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.

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 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!

6 Kit components

6.1 Components included in the kit

	Σ 96	Σ 960
REF	845-PL-0050096	845-PL-0050960
Lysis Solution CBV	60 ml	500 ml
Lysis Solution SLS	60 ml	2 x 250 ml
Precipitation Buffer P	2 x 6 ml	2 x 50 ml
MAG Suspension	5.5 ml	6 x 9 ml
Binding Solution SBS	45 ml	2 x 240 ml
Proteinase K	2 x for 1.5 ml working solution	14 x for 1.5 ml working solution
Washing Solution A	180 ml	2 x 850 ml
Washing Solution B2 (conc.)	80 ml	2 x 340 ml
RNase-Free Water	25 ml	2 x 100 ml
Manual	1	1

6.2 Components not included in the kit

- 96 %–99.8 % ethanol (molecular biology grade, undenatured)
- ddH_2O; ultrapure for dissolving Proteinase K
- 96 Flat Well plates, 96 Deep Well plate and tip combs for PP Maxi device (innuPREP Plate Set – PP Maxi (845-PLP-2000960, IST Innuscreen GmbH)
- Optionally: RNase A solution (10 mg/ml)

7 Product specifications

1. Starting material:

- Plant samples as leaves, fruits, woods, needles, seeds
- fresh, frozen or dried starting material
- max. 50 mg dry weight
- max. 100 mg wet weight

2. Time for automated extraction protocol on PurePrep Maxi:

- 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-PL-0050096/ 960	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated volume of absolute ethanol to **Washing Solution B2 (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-PL-0050096	Add 120 ml ethanol to 80 ml **Washing Solution B2**.
845-PL-00500960	Add 510 ml ethanol to 340 ml **Washing Solution B2**.

9 Sample Preparation & Homogenization

In case plant samples are not processed immediately after harvesting, they can be:

- kept at 4 °C to 8 °C for 24 hours
- lyophilized/dried within 24 hours of collection and stored at 15 °C to 30 °C
- frozen at -18 °C to -22 °C (long time storage at -78 °C to - 82 °C)
- stored in liquid nitrogen

It is recommended to collect young materials (e.g. leaves, needles) since they contain more cells per weight and therefore result in higher yields of DNA extracted. In addition, young leaves and needles contain smaller amounts of polysaccharides and polyphenols and are therefore easier to process. Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation. The lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills using ceramic beads. However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields. When using tissues other than leaves, the disruption method may require optimization to ensure maximum DNA yield and quality. After homogenization and treatment of the sample with lysis solution, the crude lysate can be cleared easily by precipitation or centrifugation.

Application example

The following example of application illustrates the effects of different lysis solutions on yield and quality of the extracted genomic DNA.

Isolation of gDNA from parsley (*Petroselinum crispum*) using the two different lysis solutions CBV and SLS. The spectrophotometric measurement shows different results depending on the lysis solution used.

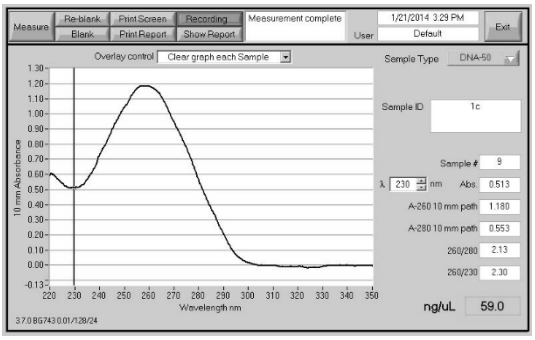

Fig. 1: Lysis Solution CBV

(Concentration: 59.0 ng/µl purity (A_{260}/A_{230}): 2.30)

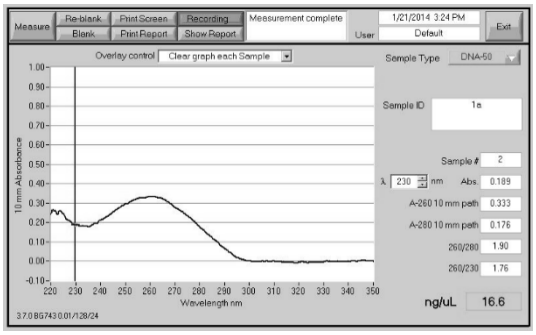

Fig. 2: Lysis Solution SLS

(Concentration: 16.8 ng/µl; purity (A_{260}/A_{230}): 1.76)

NOTE

For a large variety of plant species, either lysis solution generates good results.

9.1 Homogenization of plant material

NOTE
The lysis of the starting material is a preliminary manual processing step. To maximize the final yield of DNA a complete homogenization of plant sample is important!

1. Homogenization of about **50 - 100 mg** of **starting material** by:
   - pestle under liquid N_2 or
   - pestle in present of sand or
   - Homogenizers

2.Choosing protocol for lysis of plant material

Lysis Solution CBV	Protocol 1	(→p. 13)
Lysis Solution SLS	Protocol 2.	(→p. 13)

NOTE
To find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with the two different lysis solutions.

9.2 Protocol 1: Extraction using Lysis Solution CBV

1. Transfer the plant powder or other homogenized starting material to a 1.5 ml or 2.0 ml reaction tube. Add **500 µl Lysis Solution CBV and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.
2. Incubate at 65 °C for 30 to 60 minutes).

NOTE
We recommend using a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample.

Vortex the sample optionally 3–4 times during lysis step. No shaking will reduce the lysis efficiency.

3. Add **100 µl Precipitation Buffer P a**nd vortex the sample for 5 seconds. Incubate at room temperature for 5 minutes and centrifuge at maximum speed for 5 minutes.
4. Transfer **400 µl of lysed sample** into the DW Plate. Avoid carry-over of residual solid material!

NOTE
To remove RNA from the sample (if necessary) add 1–2 µl of RNase A solution (10 mg/ml) to the lysate, pipette up and down several times and incubate for 5 minutes at room temperature.

5. Proceed with “Automated extraction using PurePrep Maxi” on p.14.

9.3 Protocol 2: Extraction using Lysis Solution SLS

1. Transfer the plant powder or other homogenized starting material to a 1.5 ml or 2.0 ml reaction tube. Add **500 µl Lysis Solution SLS and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.

2. Incubate at 65 °C for 30 to 60 minutes.

NOTE
We recommend using a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample.

Vortex the sample optionally 3–4 times during lysis step. No shaking will reduce the lysis efficiency.

3. Centrifuge the tube at 11,000 x g for 1 minute.

4. Transfer **400 µl of lysed sample** into the DW Plate. Avoid carry-over of residual solid material!

NOTE
To remove RNA from the sample (if necessary) add 1–2 µl of RNase A solution (10 mg/ml) to the lysate, pipette up and down several times and incubate for 5 minutes at room temperature.

5. Proceed with “Automated extraction using PurePrep Maxi” on p.14

10 Automated extraction using PurePrep Maxi

10.1 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	Sample + 400 µl Binding Solution SBS + 50 µl MAG Suspension
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 B2	800 µl Washing Solution B2
Flat Well	8	Elution Plate	100 µl – 200 µl RNase-free Water

NOTE

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

10.2 Loading the PurePrep Maxi with filled plates

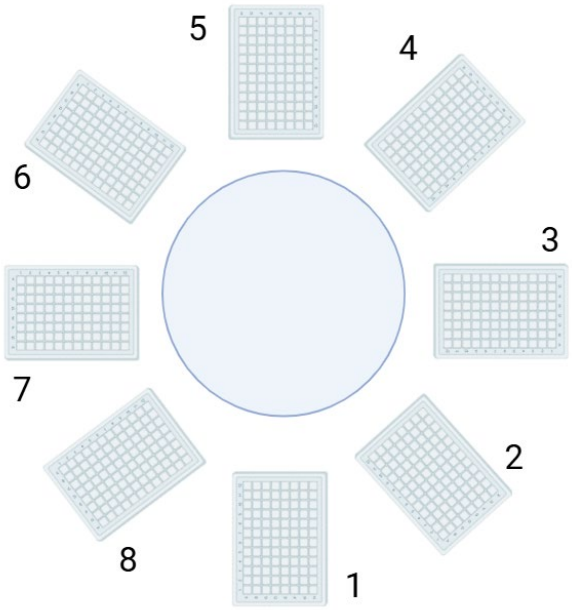

Fig. 1: Arrangement of the Plates in the device

1. Place the plates on the working table of the extraction device according to Fig.1.
2. Select the protocol “PlantDNAPP96“.
3. The automated extraction process starts with DNA binding.
4. After finishing the extraction protocol, the Flat Well Plate contains the isolated DNA.

IMPORTANT NOTE

Store the DNA under adequate conditions.

We recommend storing the extracted DNA at −22 °C to −18 °C.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Optimize homogenization process. Prolong lysis time. Reduce amount of starting material.
Low concentration of extracted DNA	
Too much RNase-free water	Elute the DNA in a lower volume of RNase-free water (min. 80 µl).
Colored eluates	
Too much starting material	Reduce amount of starting material.
Carryover of magnetic beads	
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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