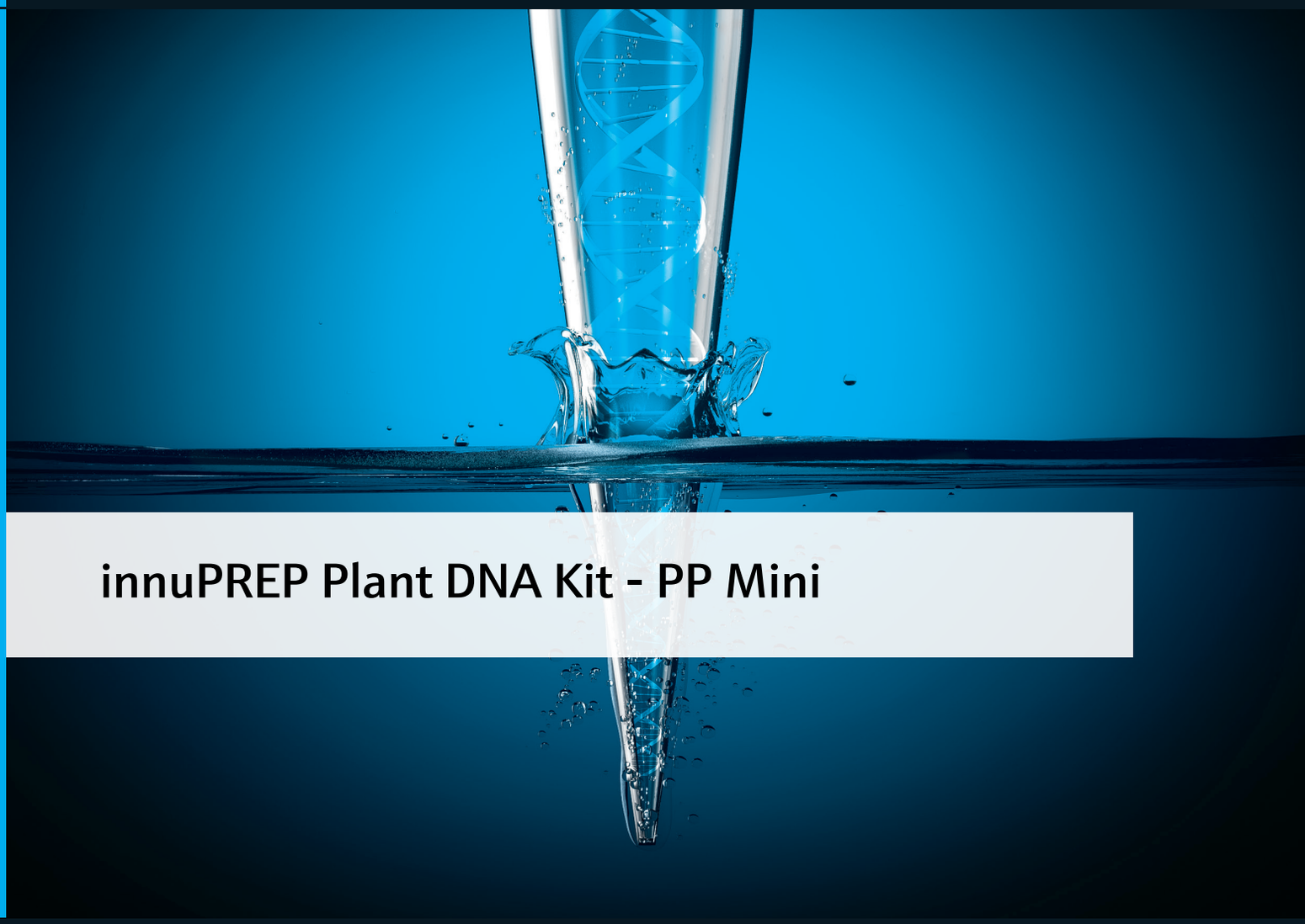
Instructions for Use
Life Science Kits & Assays



innuPREP Plant DNA Kit - PP Mini

Innuscreen GmbH

Order No.:

845-PS-0090016	16 reactions
845-PS-0090096	96 reactions

Publication No.: HB_PS-0090_e_240902

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1 Introduction

1.1 Intended use

The **innuPREP Plant DNA Kit – PP Mini** has been designed for the automated isolation of genomic DNA from plant samples on the PurePrep Mini device. The extraction procedure is based on a new kind of chemistry.

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 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 three different Lysis Solutions. Following lysis, the samples are cleared by centrifugation or precipitation.

The samples are then transferred into the DW Strip or DW Plate (available separately). The following extraction process runs automatically on the PurePrep Mini. DNA binds on the surface of the magnetic particles and after washing steps, the nucleic acid is eluted and 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 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-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 16	Σ 96
REF	845-PS-0090016	845-PS-0090096
Lysis Solution CBV	10 ml	60 ml
Lysis Solution SLS	2 x 5 ml	60 ml
Lysis Solution OPT	10 ml	2 x 25 ml
Precipitation Buffer P	2 ml	2 x 6 ml
MAG Suspension	1 ml	5.5 ml
Binding Solution SBS	8 ml	45 ml
Proteinase K	2 x for 0.3 ml working solution	2 x for 1.5 ml working solution
Washing Solution A	30 ml	180 ml
Washing Solution B2 (conc.)	16 ml	80 ml
RNase-Free Water	6 ml	25 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
- DW Strip / DW Plate / DW Tip Comb (compatible with the PP Mini device)
- 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 Mini:

- 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-0090016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-PS-0090096	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-PS-0090016	Add 24 ml ethanol to 16 ml **Washing Solution B2**.
845-PS-0090096	Add 120 ml ethanol to 80 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 (e.g. SpeedMill PLUS, Analytik Jena GmbH) 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 three different lysis solutions SLS, OPT and CBV. The spectrophotometric measurement shows different results depending on the lysis solution used.

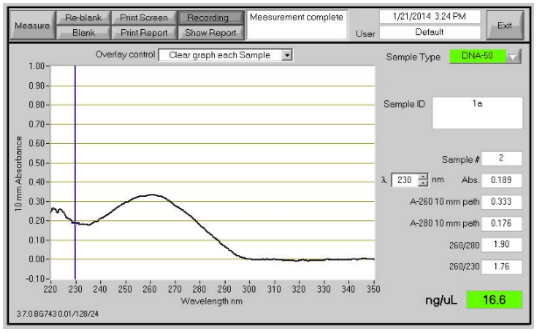

Fig. 1: Lysis Solution SLS

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

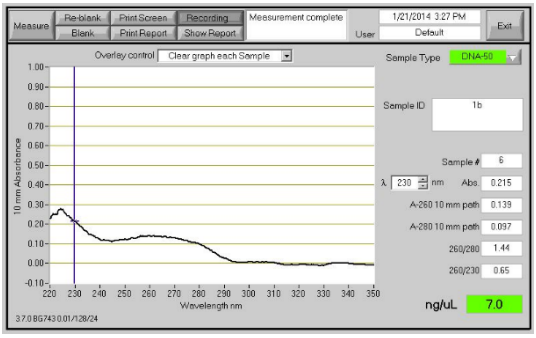

Fig. 2: Lysis Solution OPT

(yield: 7.0 ng/µl; purity (A_{260}/A_{230}): 0.65)

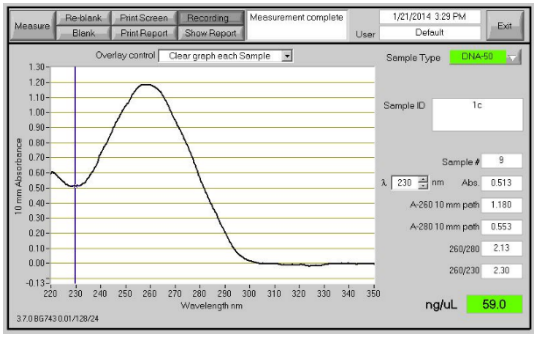

Fig. 3: Lysis Solution CBV

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

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 (e.g. SpeedMill PLUS, Analytik Jena GmbH)

2. Choosing protocol for lysis of plant material

Lysis Solution SLS	Protocol 1	(→p. 12)
Lysis Solution OPT	Protocol 2	(→p. 13)
Lysis Solution CBV	Protocol 3.	(→p. 14)

NOTE
In order 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 three different lysis solutions.

9.2 Protocol 1: 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 first cavity of DW Strip or 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 Mini” on p.15.

9.3 Protocol 2: Extraction using Lysis Solution OPT

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 OPT**, 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** and 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 first cavity of DW Strip or 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 Mini” on p.15.

9.4 Protocol 3: 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 first cavity of DW Strip or 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 Mini” on p.15.

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. 4: Schematic illustration of DW Plate

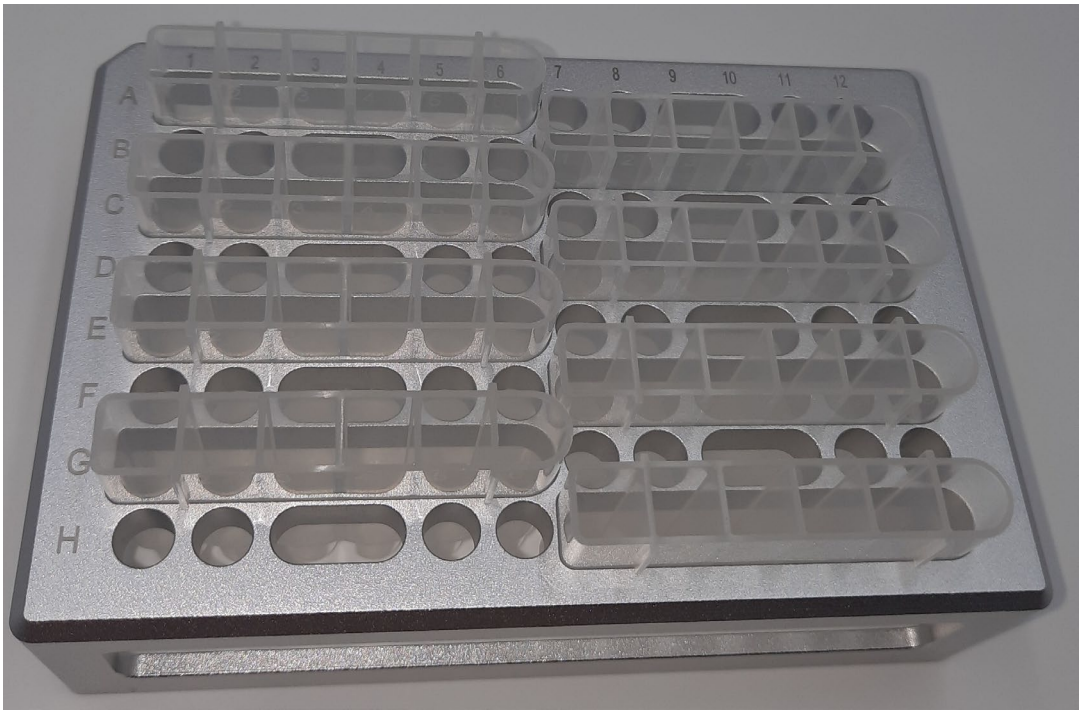

Fig. 5: Arrangement of the DW Strips in Tray

Cavity of DW Plate/Strip	Content
Cavity 1	Sample + 400 µl Binding Solution SBS + 50 µl MAG Suspension
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 B2
Cavity 6	100 µl – 200 µl RNase-free Water

The prefilling is carried out from left to right as shown in the illustration, Fig. 4. 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

   "PlantDNA" and start the run.
2. The automated extraction process starts with DNA binding.
3. After finishing the extraction protocol, the Cavity 6 and 12 contains the isolated DNA.
4. Transfer the DNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

Store the DNA under adequate conditions.

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

If the 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.

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 Elution Buffer	Elute the DNA in a lower volume of Elution Buffer (min. 80 µl).
Colored eluates	
Too much starting material	Reduce amount of starting material.

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