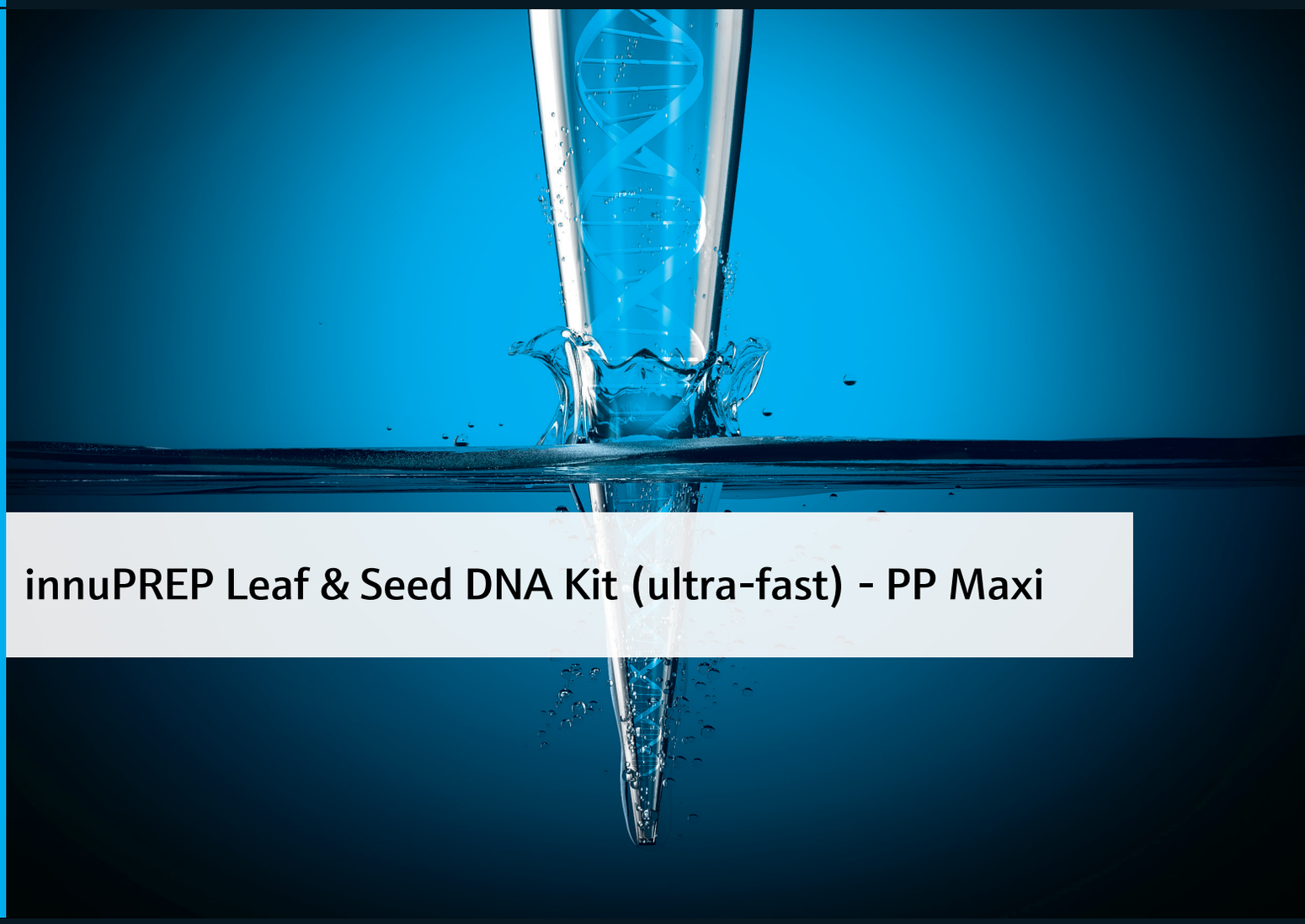
Instructions for Use
Life Science Kits & Assays



innuPREP Leaf & Seed DNA Kit (ultra-fast) - PP Maxi

Innuscreen GmbH

Order No.:

845-PL-0010096	96 reactions
845-PL-0010960	960 reactions
845-PL-0019600	9600 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

Manufacturer and Distributor:
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

Contents

1 Introduction

1.1 Intended use

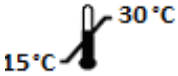
The **innuPREP Leaf & Seed DNA Kit (ultra-fast)– PP Maxi** has been designed for the ultra-fast automated isolation of DNA from plant material using the PurePrep Maxi device.

The kit allows extremely fast extraction of DNA from plant leaves and seeds. Based on a novel extraction chemistry in combination with paramagnetic particles, the use of classic chaotropic high-salt buffers as well as washing buffers containing no ethanol or isopropanol. This makes it possible to extract DNA in high-throughput format without the need for large quantities of flammable liquids in the laboratory. The quality and quantity of the extracted DNA is excellent and allows the DNA to be used for a wide range of downstream applications.

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.
 N	**Content** Contains sufficient reagents for <N> reactions.
	**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 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. →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 General notes for isolation of DNA from plants

1. The homogenization of the sample is an essential step. We recommend the use of commercial homogenizers (e.g. Tissue Homogenizer 2010 Geno/Grinder).

2. As starting material for DNA extraction 20 – 50 mg are sufficient.

3. Depending on plant species the volume of Lysis Solution can be increased. For oil-rich plants we recommend using less starting material or increasing the volume of Lysis Solution CBV.

4. If samples contain large amounts of RNA, we recommend adding RNase A (10 mg/ml) to the lysis mixture.

4 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 **innuPREP Leaf & Seed DNA Kit (ultra-fast)– PP Maxi** 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.

5 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 Leaf & Seed DNA Kit (ultra-fast)– 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.com. For other countries please contact your local distributor.

6 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 (→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!

7 Kit components

7.1 Components included in the kit

	Σ 96	Σ 960	Σ 9600
REF	845-PL-0010096	845-PL-0010960	845-PL-0019600
MAG Suspension F	1.1 ml	6 x 1.1 ml	60 ml
Lysis Solution CBV	60 ml	600 ml	6 l
Precipitation Buffer P	6 ml	50 ml	500 ml
Binding Solution SBS	15 ml	150 ml	2 x 1 l
Proteinase K	2 x 6 mg (for 0.3 ml working solution)	4 x 30 mg (for 1.5 ml working solution)	2 x 600 mg (for 30 ml working solution)
Washing Solution P	60 ml	600 ml	6 l
Washing Solution ER	30 ml	300 ml	3 l
Manual	1	1	1

7.2 Components not included in the kit

- Homogenization Plate or innuSPEED Lysis Tubes (for grinding of plants or seeds)
- ddH_2O; ultrapure for dissolving Proteinase K
- RNase-free water for elution step
- 96 FlatWell plates, 96 DeepWell plate and tip combs for PP Maxi device (845-PLP-1000960 innuPREP Plate Set – PP Maxi, IST Innuscreen GmbH)

8 Product Specifications

8.1 Starting material:

- 20 – 50 mg of homogenized plant or seed material

8.2 Time for isolation

- Approximately 10 min for automated extraction

9 Initial Steps before starting

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

845-PL-0010096	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-PL-0010960	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-PL-0019600	Add 30 ml ddH_2O to lyophilized Proteinase K.

- Avoid freezing and thawing of starting material.
- Prepare **Lysis Solution CBV / Proteinase K** according to the special protocols.

V1: for 300 µl Lysis Solution CBV/rxn

Component	**1 sample**	**96 samples** (+20% dead volume compensation)
Lysis Solution CBV	300 µl	35 ml
Proteinase K	5 µl	580 µl
Final volume		35.6 ml
Volume to be added		305 µl/rxn

V2: for 350 µl Lysis Solution CBV/rxn

Component	**1 sample**	**96 samples (+20% dead volume compensation)**
Lysis Solution CBV	350 µl	40 ml
Proteinase K	5 µl	580 µl
Final volume		40.6 ml
Volume to be added		355 µl/rxn

V3: for 500 µl Lysis Solution CBV/rxn

Component	1 sample	96 samples (+20% dead volume compensation)
Lysis Solution CBV	500 µl	58 ml
Proteinase K	5 µl	580 µl
Final volume		58.6 ml
Volume to be added		505 µl/rxn

- Prepare **Binding Solution SBS / MAG Suspension F** according to the special protocols.

Component	n samples	96 samples (+20% dead volume compensation)
Binding Solution SBS	120 µl	14 ml
MAG Suspension F	5 µl	580 µl
Final volume		14.6 ml
Volume to be added		125 µl

NOTE
Mix the **MAG Suspension F** well by vortexing for 1 minute. To prevent the MAG particles from settling, please prepare the **Binding Solution SBS / MAG Suspension F** mix for only one plate (96 rxn) at a time and do not leave it to stand for a long time.

10 Sample Preparation

10.1 Homogenization

1. Use 20 – 50 mg of sample material and transfer it into a “Grinding Plate” (96-well-plate or 96 array sample tube rack with steel beeds). Alternatively refer to our innuSPEED Lysis Tubes.

2. Homogenize the starting material using a commercial Grinder (e.g.Tissue homogenizer 2010 Geno/Grinder®, SpeedMill, FastPrep).

10.2 Lysis

1. Add Lysis Solution CBV (volume depends on starting material, see table below) and add 5 µl Proteinase K to the powdered material.

Sample Material	Volume CBV [µl]
Seed powder	350
Seed powder from oil-rich seeds	500
Plant leaf powder (fresh & dried material)	300

NOTE

A. For oil-rich plants we recommend using no more than 30 mg.

B. For large number of samples prepare a “Master mix” of Lysis Solution and Proteinase K.

C. Optional add 2 µl RNase A (10mg/ml) per sample to the lysis mix.

2. Incubate the Plate at 65˚C under continuously shaking for 20 – 30 minutes.

3. Add:
   - 15 µl Precipitation Buffer P for leaf samples or
   - 20 µl Precipitation Buffer P for seed

   Vortex shortly and centrifuge at minimum 2.500 x g for 10 minutes.

4. Carefully transfer 120 µl of the cleared supernatant into a 96-FlatWell Plate (labeled with “Binding Plate”).

11 Automated Extraction using PurePrep Maxi

11.1 Prefilling of the Plates

Label and fill the 96 Well plates according to the table below.

Plate	Label	Content
FlatWell 1	Binding	120 µl supernatant of lysed sample + 125 Binding Solution SBS/ MAG Suspension F Mastermix
FlatWell 2	Washing P	250 µl Washing Solution P
FlatWell 3	Washing P	250 µl Washing Solution P
FlatWell 4	Washing ER	250 µl Washing Solution ER
FlatWell 5	Elution	100 – 150 µl RNase-free Water
DeepWell 1	Tip Comb Plate	96 Well Tip Comb

11.2 Loading filled Plates to the PurePrep Maxi

1. Turn on and select the protocol “MSEED1” on PurePrep Maxi instrument and start the run.
2. Follow the instructions and load the filled plates to the right tray position according to the instruction of the device.
3. After finishing the extraction protocol all Flat-well plates can be removed. The Deep-Well Plate in position 1 should be reused for the next run.

IMPORTANT NOTE

After finishing the extraction protocol, the Elution Plate contains the isolated DNA. Store the DNA under adequate conditions. For long time storage we recommend storing the extracted DNA 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.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Poor lysis of starting material	
Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no DNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!

IST Innuscreen GmbH
Robert-Rössle-Str.10
13125 Berlin · Germany

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

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