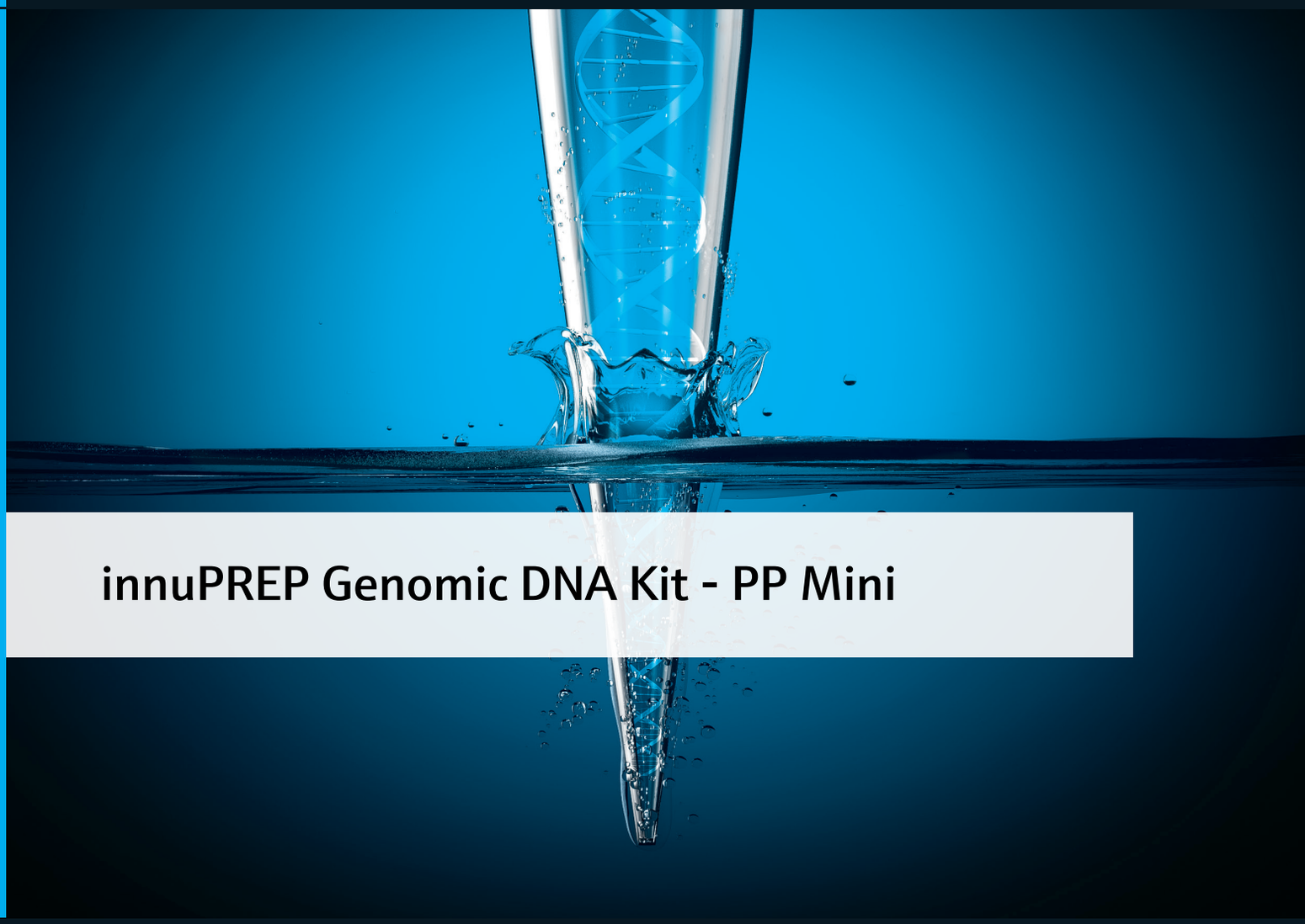
Instructions for Use
Life Science Kits & Assays



innuPREP Genomic DNA Kit - PP Mini

iST Innuscreen GmbH

Order No.:

845-PS-0020016	16 reactions
845-PS-0020096	96 reactions

Publication No.: HB_PS-0020_e_230705

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 Genomic DNA Kit – PP Mini** has been designed for the automated isolation of genomic DNA from different kinds of starting material on the PurePrep Mini device. The extraction procedure is based on a new kind of chemistry. The kit can be used for isolation of genomic DNA from whole blood samples (100 µl), buffy coat (100 µl), dry swabs, swabs in storage buffer, tissue samples (up to 20 mg), rodent tails, semen, saliva, hair/hair roots.

The extraction procedure takes place on the magnetic particle processor PurePrep Mini and allows the parallel and flexible extraction of up 1 to 16 samples.

The extraction process starts with sample lysis (external step) followed by automated extraction of gDNA on the PurePrep Mini.

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, 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 Genomic 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-0020016	845-PS-0020096
Lysis Solution CBV	10 ml	60 ml
MAG Suspension F	0.25 ml	1.1 ml
Binding Solution SBS	8 ml	45 ml
Proteinase K	2 x 0,3 ml	2 x 1,5 ml
Washing Solution A	30 ml	180 ml
Washing Solution B2 (conc.)	10 ml	48 ml
Washing Solution ER	17 ml	85 ml
Elution Buffer	6 ml	25 ml
Manual	1	1

6.2 Components not included in the kit

- 1.5 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)
- DTT (1M, optional)

7 Product specifications

1. Starting material:

- Whole blood samples (100 µl)
- Buffy coat (100 µl)
- Dry swabs
- Swabs in storage buffer
- Tissue samples, rodent tails (up to 20 mg)
- Semen
- Saliva
- Hair/Hair roots
- etc.

2. Time for automated extraction protocol on PurePrep Mini:

- Approx. 20 minutes (excluding external lysis)

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-0020016	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-PS-0020096	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-0020016	Add 15 ml ethanol to 10 ml **Washing Solution B2**.
845-PS-0020096	Add 72 ml ethanol to 48 ml **Washing Solution B2**.

9 Sample Preparation

9.1 Protocol 1: Isolation of DNA from whole blood or buffy coat

1. Transfer 100 µl whole blood or buffy coat in a 1.5 ml tube.
2. Add **300 µl Lysis Solution CBV** and **20 µl Proteinase K** to the blood sample or 30 µl Proteinase K to the buffy coat sample. Vortex shortly.
3. Incubate at 55°C under continuously shaking conditions for 30 minutes.
4. Proceed with “Automated extraction using PurePrep Mini” on p.13.

NOTE
If the volume of the blood sample is less than 100 µl adjust with PBS to 100 µl.

9.2 Protocol 2: Isolation of DNA from tissue / rodent tail (5 – 20 mg)

1. Cut the tissue sample / rodent tail (5-20 mg) in small pieces and transfer it in a 1.5 ml tube.
2. Add **400 µl Lysis Solution CBV** and **30 µl Proteinase K** to the sample. Vortex shortly.
3. Incubate at 55°C under continuously shaking conditions for minimum 90 minutes.
4. After lysis centrifuge the tube for 5 minutes at maximum speed. Use the clear supernatant for the following automated extraction.
5. Proceed with “Automated extraction using PurePrep Mini” on p.13.

9.3 Protocol 3: Isolation of DNA from Swabs

A. Swabs in storage buffer

1. Squeeze out the swab and remove the swab.
2. Transfer **200 µl** of the storage buffer into a 1.5 ml tube.
3. Add **200 µl Lysis Solution CBV** and **20 µl Proteinase K** to the sample. Vortex shortly.
4. Incubate at 55°C under continuously shaking conditions for 30 minutes.
5. Proceed with "Automated extraction using PurePrep Mini" on p.13.

B. Dry Swabs

1. Place the swabs into tubes containing **500 µl Lysis Solution CBV** and incubate the swab for 10 minutes.
2. Squeeze out the swab and remove the swab.
3. Add **20 µl Proteinase K** and vortex shortly.
4. Incubate at 55°C under continuously shaking conditions for 30 minutes.
5. Proceed with "Automated extraction using PurePrep Mini" on p.13.

9.4 Protocol 4: Isolation of DNA from hair/hair roots

1. Cut the hair in small pieces and transfer it into a 1.5 ml tube.
2. Add **400 µl Lysis Solution CBV** to the cut hair sample.
3. Add **20 µl Proteinase K** and **30 µl DTT solution (1 M)** (not provided). Vortex shortly.
4. Incubate at 55 °C under continuously shaking conditions for at least 3 hours.
5. Proceed with "Automated extraction using PurePrep Mini" on p.13.

9.5 Protocol 5: Isolation of DNA from sperm samples

1. Transfer 100 µl sperm sample into a 1.5 ml tube.
2. Add **300 µl Lysis Solution CBV** to the sperm sample.
3. Add **20 µl Proteinase K** and **30 µl DTT solution (1 M)** (not provided). Vortex shortly.
4. Incubate at 55 °C under continuously shaking conditions for at least 3 hours.
5. Proceed with “Automated extraction using PurePrep Mini” on p.14.

9.6 Protocol 6: Isolation of DNA from Saliva

A. Saliva

1. Transfer 200 µl saliva sample into a 1.5 ml tube.
2. Add **200 µl Lysis Solution CBV** and **20 µl Proteinase K**. Vortex shortly.
3. Incubate at 55°C under continuously shaking conditions for 30 minutes.
4. Proceed with “Automated extraction using PurePrep Mini” on p.14.

B. Saliva in transportation medium

1. Transfer 200 µl of sample (medium) into a 1.5 ml tube.
2. Add **200 µl Lysis Solution CBV** and **20 µl Proteinase K**. Vortex shortly.
3. Incubate at 55°C under continuously shaking conditions for 30 minutes.
4. Proceed with “Automated extraction using PurePrep Mini” on p.13.

10 Automated extraction using PurePrep Mini

10.1 Prefilling of the DW Plate or the DW Strips

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 A
Cavity 3	800 µl Washing Solution A
Cavity 4	800 µl Washing Solution B2
Cavity 5	800 µl Washing Solution ER
Cavity 6	100 µl – 200 µl Elution Buffer (volume depends on starting material and expected yield)

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.
- The tip combs always dip staggered into the Strips.

  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

   "GDNA1" and start the run.
2. After finishing the extraction protocol, the Cavity 6 contains the isolated DNA.
3. Transfer the DNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

After finishing the extraction protocol, the Elution Plate contains the isolated DNA. Store the DNA under adequate conditions.

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

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

IST Innuscreen GmbH