

Instructions for Use

Life Science Kits & Assays



innuPREP Genomic DNA Kit - PP Mini

Order No.:

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

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It needs not necessarily agree with future versions. Subject to change!

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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 1 up 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.



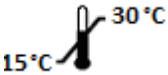





CONSULT INSTRUCTION FOR USE



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 Catalogue number.
	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. → „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 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

	 16	 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
RNase-free Water	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)
- ddH₂O; ultrapure for dissolving Proteinase K
- DTT (1M, optional)
- innuPREP Plate Set - PP Mini, IST Innuscreen GmbH, 845-PSP-1000016/845-PSP-1000096 (suitable for processing up to 16 samples simultaneously)
- innuPREP Strip Set – PP Mini, IST Innuscreen GmbH, 845-PSS-2000016/845-PSP-2000096 (suitable for flexible processing of 1 up to 8 samples simultaneously)

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
- Cells (prokaryotic & eukaryotic)

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 ddH₂O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-PS-0020016	Add 0.3 ml ddH ₂ O to lyophilized Proteinase K.
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845-PS-0020096	Add 1.5 ml ddH ₂ O to lyophilized Proteinase K.
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- 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.
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845-PS-0020096	Add 72 ml ethanol to 48 ml Washing Solution B2.
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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 the PurePrep Mini" on p.14.

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 the PurePrep Mini" on p.14.

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 μ l of the storage buffer into a 1.5 ml tube.
3. Add 200 μ l Lysis Solution CBV and 20 μ l 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 the PurePrep Mini" on p.14.

B. Dry Swabs

1. Place the swabs into tubes containing 500 μ l Lysis Solution CBV and incubate the swab for 10 minutes.
2. Squeeze out the swab and remove the swab.
3. Add 20 μ l Proteinase K and vortex shortly.
4. Incubate at 55°C under continuously shaking conditions for 30 minutes.
5. Proceed with "Automated Extraction using the PurePrep Mini" on p.14.

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 μ l Lysis Solution CBV to the cut hair sample.
3. Add 20 μ l Proteinase K and 30 μ l 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 the PurePrep Mini" on p.14.

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 the 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.
1. Proceed with "Automated Extraction using the PurePrep Mini" on p.14.

B. Saliva in transportation medium

2. Transfer 200 µl of sample (medium) into a 1.5 ml tube.
3. Add 200 µl Lysis Solution CBV and 20 µl Proteinase K. Vortex shortly.
4. Incubate at 55°C under continuously shaking conditions for 30 minutes.

5. Proceed with "Automated Extraction using the PurePrep Mini" on p.14.

9.7 Protocol 7: Isolation from cells (prokaryotic & eukaryotic)

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g). Discard the supernatant.
2. Resuspend the cell pellet in **200 µl TE- or PBS-Buffer**.

NOTE

In some cases if you have bacteria which are hard to lyse please refer additionally to our innuPREP Bacteria Lysis Booster or for yeasts to Lyticase.

3. Add **200 µl Lysis Solution CBV** and **20 µl Proteinase K**. Vortex shortly.
4. Incubate at 55°C under continuously shaking conditions for 30 minutes.
5. Proceed with "Automated Extraction using the PurePrep Mini" on p.14.

10 Automated Extraction using the 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

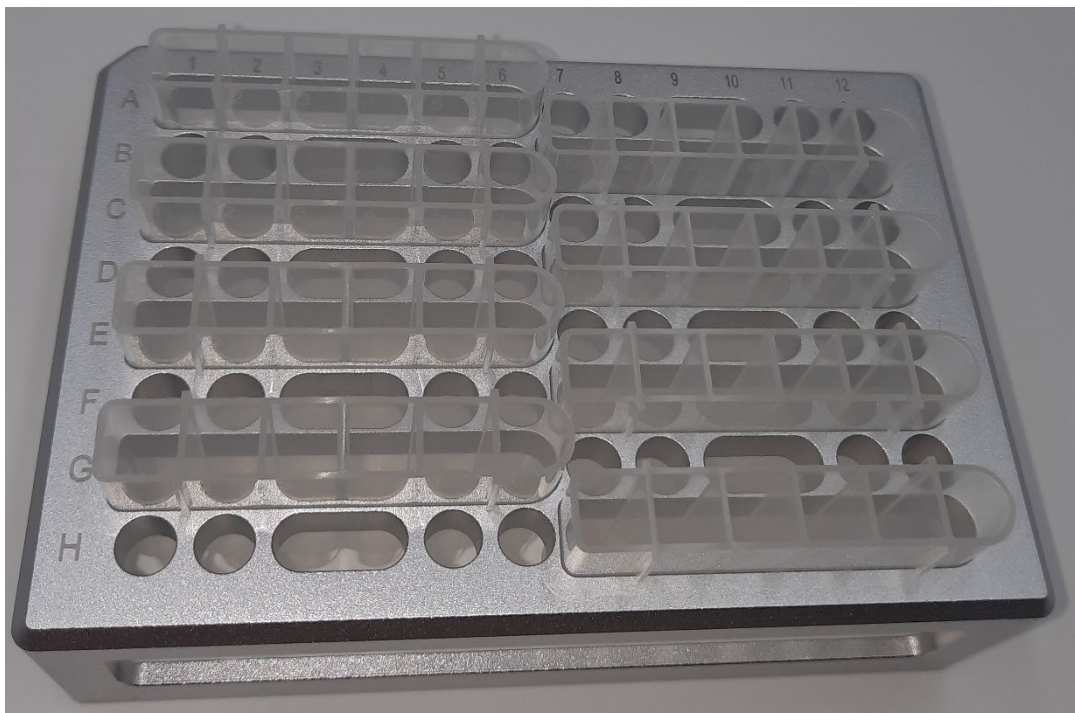


Fig. 2: Arrangement of the DW Strips in Tray

Cavity of 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 RNase-free Water (volume depends on starting material and expected yield)

The prefilling is carried 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 strip (strips), only every second tip is being used for extraction:

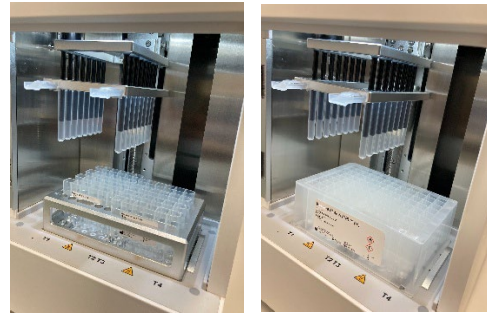
Left tray side: Tip 1, 3, 5, 7

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

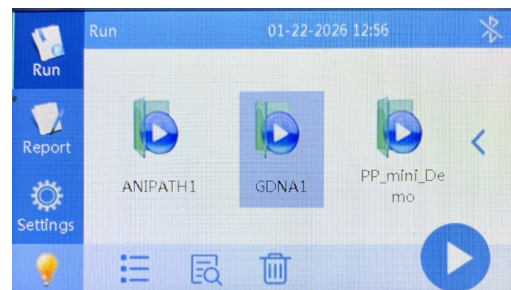
Automated Extraction using the PurePrep Mini

1. Place the Plate and Strips inserted in the Holder (tray) into the PupePrep Mini with the label facing outward.
2. Insert the tip comb in the tip comb holder.



Reagent Strips Reagent Plate

3. Select the protocol "GDNA1" and start the run.



4. After finishing the extraction protocol, the Cavities 6 and 12 contain the isolated DNA.
5. Transfer the DNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

After finishing the extraction protocol, the last cavity of the Plate/Strip 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 .

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 RNase-free Water	Elute the DNA in a lower volume of RNase-free Water (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 DNA into a new plate or Elution vessels

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