

Instructions for Use

Life Science Kits & Assays



innuPREP Blood DNA Kit - PP Mini, prefilled

Order No.:

845-PPA-1670016	16 reactions
845-PPA-1670096	96 reactions
845-PPB-1670016	16 reactions
845-PPB-1670096	96 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!

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1 Introduction

1.1 Intended use

The **innuPREP Blood DNA Kit – PP Mini, prefilled** has been designed for the automated isolation of genomic DNA from whole blood on the PurePrep Mini device. The extraction procedure is based on a new kind of chemistry.

The Reagent Plate / Strip of the kit is prefilled with almost all extraction reagents which are needed for the extraction process. After addition of the whole blood sample and the Proteinase K, the extraction procedure begins with sample lysis. In between the device stops and MAG Suspension F and Binding Solution SBS must be added to the lysed sample. Afterwards Reagent Plate / Strip must be put back to the device and the automated extraction is followed by binding of DNA to surface-modified magnetic particles. After washing steps, the DNA is eluted from the magnetic particles with Elution Buffer and is now ready to use for downstream applications.

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. Additionally, the kit is compatible with the PurePrep Midi device. Please refer to the corresponding chapters of the devices.

The kit is intended for use by professional users. The DNA extracted using this kit is suitable for a wide range of downstream applications, such as amplification reactions and further analytical procedures.



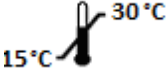







CONSULT INSTRUCTION FOR USE

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“ 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 Blood DNA Kit – PP Mini, prefilled** 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-PPA-1670016 ^a 845-PPB-1670016 ^b	845-PPA-1670096 ^a 845-PPB-1670096 ^b
MAG Suspension F	0.25 ml	1.1 ml
Proteinase K	2 x for 0.3 ml working solution	2 x for 1.5 ml working solution
Binding Solution SBS	8 ml	30 ml
Reagent Plate A (PP) ^a	1	6
Reagent Strip A (PP) ^b	16	96
Tip Combs ^a	2	12
Tip Combs ^b	4	24
Manual	1	1

6.2 Components not included in the kit

- ddH₂O; ultrapure for dissolving Proteinase K
- optional further Tip Combs (12 pcs)(31-01888, IST Innuscreen)

7 Product specifications

1. Starting material:

- Whole blood samples (200 µl)
- Fresh or frozen whole blood samples
- Stabilized with EDTA or Citrate

2. Time for automated extraction protocol on PurePrep Mini:

- Appr. 58 minutes

Time for automated extraction protocols on PurePrep Midi:

- Lysis: 25 minutes
- Extraction: 30 minutes

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-PPA-1670016

845-PPB-1670016

Add 0.3 ml ddH₂O to lyophilized Proteinase K.

845-PPA-1670096

845-PPB-1670096

Add 1.5 ml ddH₂O to lyophilized Proteinase K.

9 Preparing Reagent Plate / Strip for automated extraction

9.1 General filling scheme of the reagents (already prefilled)

Cavity of DW Plate/Strip	Content
Cavity 1	300 µl Lysis Solution CBV (2x)
Cavity 2	800 µl Washing Solution A
Cavity 3	800 µl Washing Solution B2
Cavity 4	800 µl Washing Solution B2
Cavity 5	empty
Cavity 6	200 µl Elution Buffer

9.2 Unpacking of Reagent Plate and peeling off the sealing foil



Reagent Plates and Reagent Strips are delivered wrapped into plastic bags for transport protection.

Carefully open the overpack of Reagent Plates/Strips by using scissors.

Afterwards gently remove the foil by peeling off.

10 Sample Preparation

10.1 Isolation from 200 µl whole blood samples

1. Add 200 µl of the whole blood sample and 20µl Proteinase K into the first well of the Deep Well Plate/Strip.

NOTE

If the volume of the blood sample is less than 200 µl adjust with PBS to 200 µl.

3. Proceed with "Automated extraction using PurePrep Mini" on p.11. or "Automated extraction using PurePrep Midi" on p. 14.

11 Automated extraction using PurePrep Mini

11.1 Prefilled 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

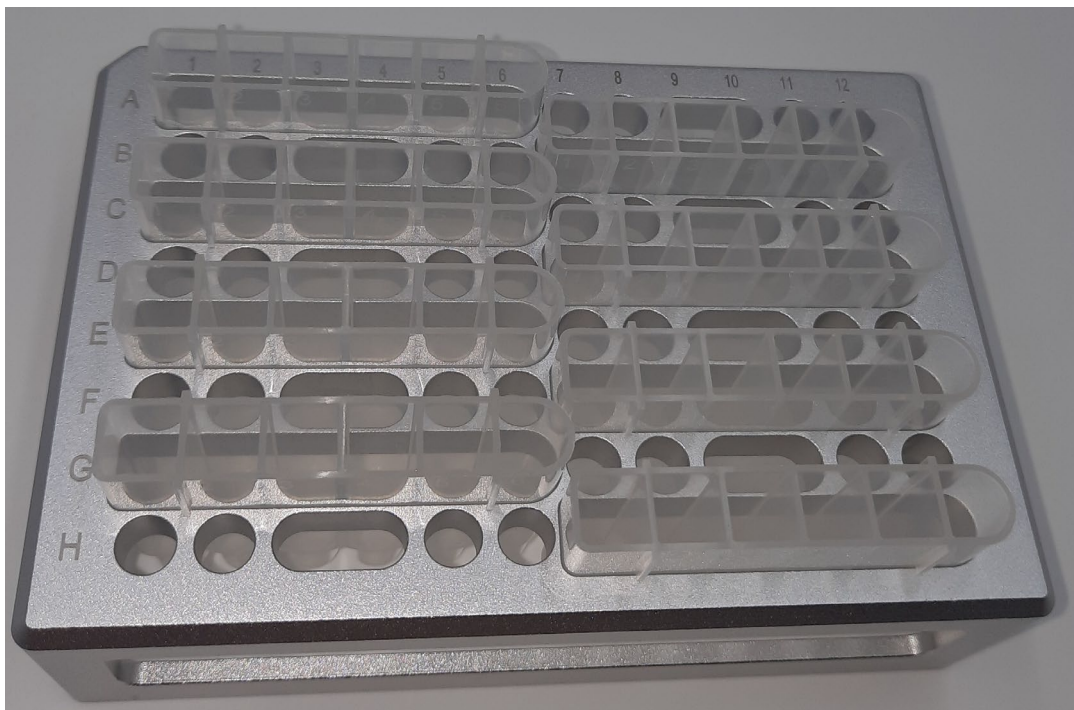


Fig. 2: Arrangement of the DW Strips in Tray

11.2 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
"BLOODDNA" and start the run.
 2. The automated extraction process starts with sample lysis. After sample lysis the automated run stops.
 3. After the device has stopped, take the Plate/Strip out of the device and add 5 µl of well mixed **MAG Suspension F** and 285 µl of **Binding Solution SBS** to the lysed samples in cavity 1 and/or 7.
-

NOTE

Mix the **MAG Solution F** well by vortexing for 1 minute.

4. After addition of **MAG Suspension F** and **Binding Solution SBS** place the Plate/Strip back to the PurePrep Mini and continue the extraction process by starting the device (you will find the instruction on the display of the PurePrep Mini).
5. After finishing the extraction protocol, the Cavities 6 and 12 contain the isolated DNA.
6. Transfer the DNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

After finishing the extraction protocol, cavities 6 and 12 contain the isolated DNA. Store the DNA under adequate conditions.

We recommend storing the extracted DNA for longer use at $-22\text{ }^{\circ}\text{C}$ to $-18\text{ }^{\circ}\text{C}$.

12 Automated extraction using PurePrep Midi

NOTE

According to the chapter of the PurePrep Mini the illustration of the plates will be the same!

The prefilling is carried out from left to right as shown in the illustration, Fig. 1. For the PurePrep Midi, 2 DW Plates can be loaded in parallel to run 32 samples.

12.1 Loading filled Deep Well Plate to the PurePrep Midi and plug in the Tip Combs

NOTE

It is recommended to mark the tips used for the extraction so that they are not used more than once.

1. Select the protocol "BloodLysisPP32" and start the run.
 2. The automated extraction process starts with sample lysis. After sample lysis the automated run stops.
 3. After the device has stopped, take the Plate(s) out of the device and add **5 µl** of well mixed **MAG Suspension F** and **285 µl** of **Binding Solution SBS** to the lysed samples in cavity 1 and 7.
-

NOTE

Mix the **MAG Solution F** well by vortexing for 1 minute.

4. Place the Plate(s) back to the PurePrep Midi and select the protocol "BloodPP32" and start the run.
5. After finishing the extraction protocol, the Cavity 6 / 12 contains the isolated DNA.
6. Transfer the DNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

After finishing the extraction protocol, cavities 6 and 12 of plate 1 and/or 2 contain the isolated DNA. Store the DNA under adequate conditions.

We recommend storing the extracted DNA for longer use at $-22\text{ }^{\circ}\text{C}$ to $-18\text{ }^{\circ}\text{C}$.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
NO extracted DNA	No Magnetic beads or no Binding Solution SBS are added to cavity 1 and/or 7 after Lysis step. Ensure MAG Suspension F has been mixed well before use.
Insufficient lysis of starting material	Ensure that Proteinase K is added in the Cavity 1 and /or in the Cavity 7
Inadequate extraction	Inhibiting substances in starting material. Please use the kit only for samples that match the requirements declared in "Product specifications". Use internal controls for verification of extraction procedure
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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