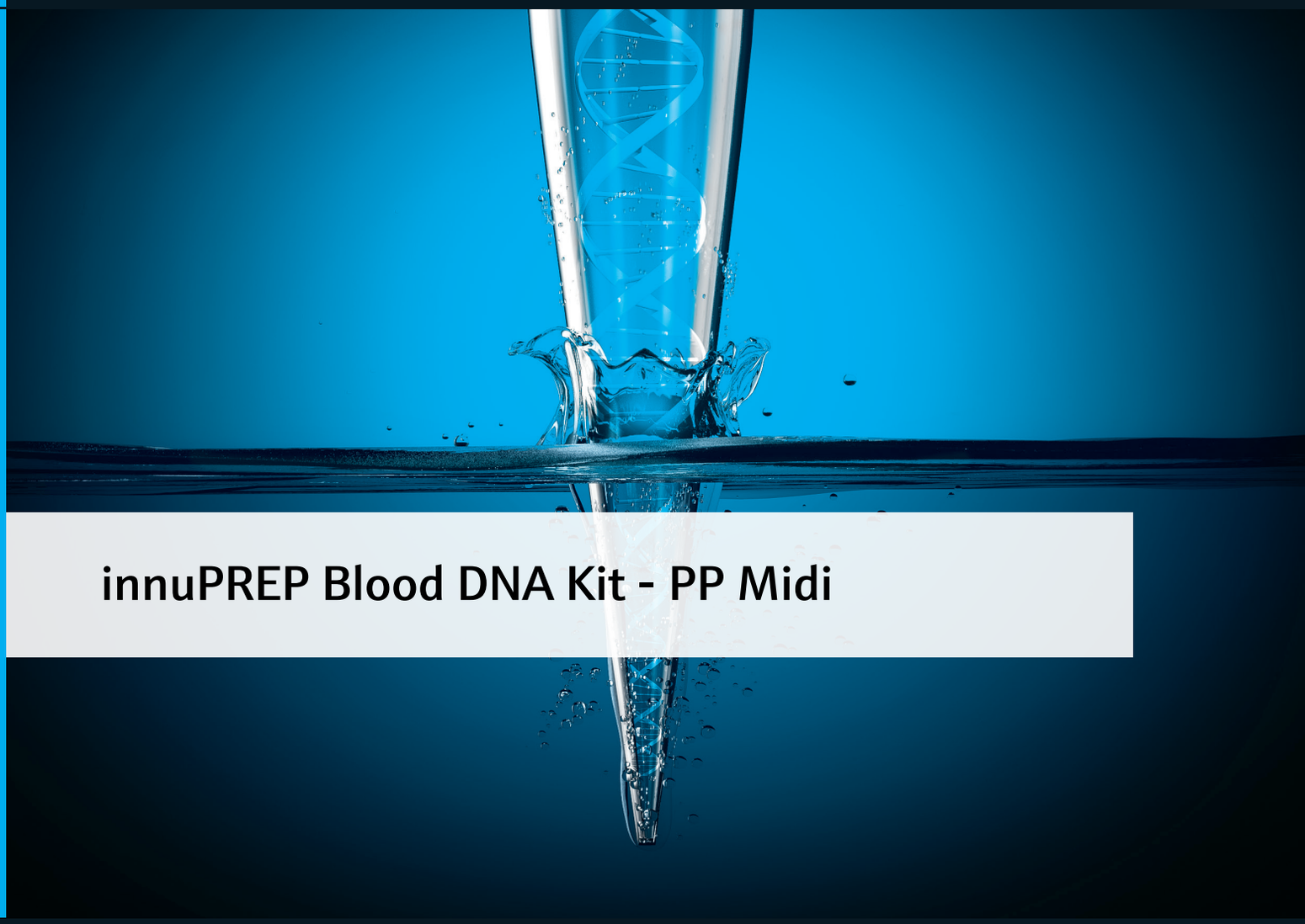
Instructions for Use
Life Science Kits & Assays



innuPREP Blood DNA Kit - PP Midi

Innuscreen GmbH

Order No.:

845-PM-0020032	32 reactions
845-PM-0020192	192 reactions

Publication No.: HB_PM-0020_e_250625

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

The extraction procedure takes place on the magnetic particle processor PurePrep Midi and allows the parallel and flexible extraction from 1 up to 32 samples.

The extraction process starts with sample lysis on the PurePrep Midi followed by binding of DNA on the surface of the magnetic particle. After washing the DNA is eluted in Elution Buffer.

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 can 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 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 Midi** 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. 7). 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 32	Σ 192
REF	845-PM-0020032	845-PM-0020192
Lysis Solution CBV (2x)	12 ml	60 ml
MAG Suspension F	0.25 ml	1.1 ml
Binding Solution SBS	12 ml	60 ml
Proteinase K	1.5 ml	3 x 1.5 ml
Washing Solution A	30 ml	180 ml
Washing Solution B2 (conc.)	24 ml	136 ml
Elution Buffer	7 ml	50 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
- 96-u-well-plates and tip combs for PurePrep Midi (alternative innuPREP Plate Set - PP Midi)

7 Product specifications

1. Starting material:

- Whole blood samples (200 µl)

2. Time for automated extraction protocol on PurePrep Midi:

- Lysis: 25 minutes
- Extraction: 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-PM-0020032	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-PM-0020192	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-0020032	Add 36 ml ethanol to 24 ml **Washing Solution B2**.
845-PS-0020192	Add 204 ml ethanol to 136 ml **Washing Solution B2**.

9 Sample Preparation

9.1 Isolation from 200 µl whole blood samples

1. Transfer **200 µl** of the whole blood sample into the first well of the Deep Well Plate.
2. Add **300 µl Lysis Solution CBV (2x)** and add **20 µl Proteinase K** to each well used.

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 Midi” on p.130.

10 Automated extraction using PurePrep Midi

IMPORTANT

Make sure Washing Solution B2 (conc.) as well as Proteinase K have been prepared as indicated (refer to p. 8 “Initial steps before starting”).

10.1 Prefilling of the DW Plate

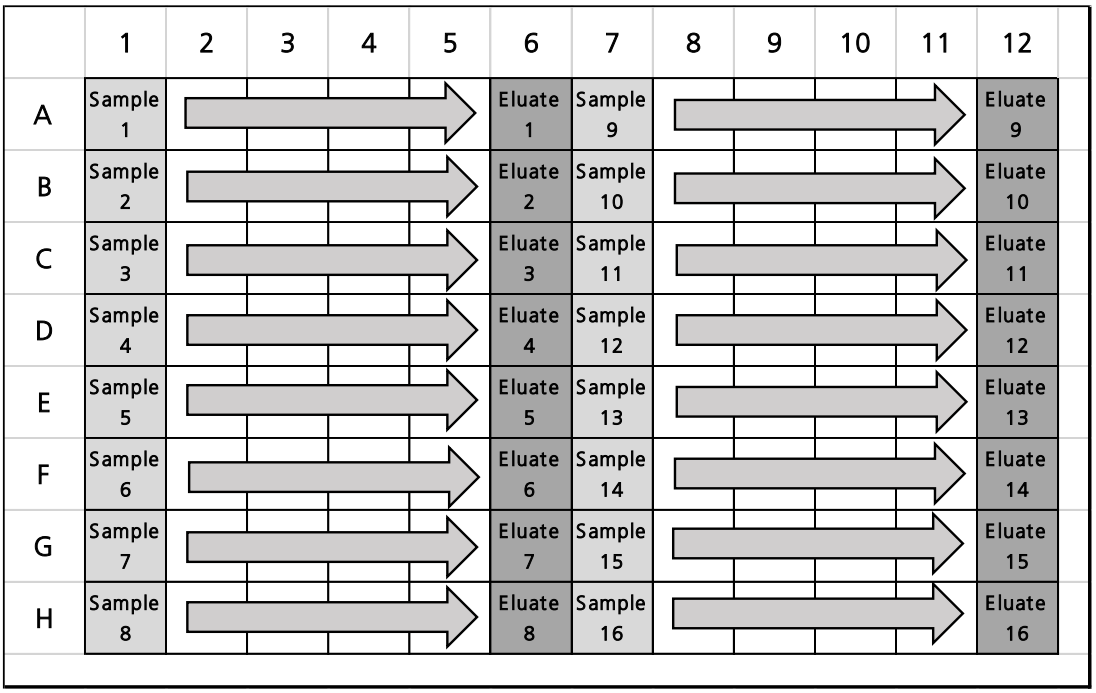


Fig. 1: Schematic illustration of DW Plate

Cavity of DW Plate	Content
Cavity 1 + 7	Sample + 300 µl Lysis Solution CBV (2x) + 20 µl Proteinase K (refers to step 9.1)
Cavity 2 + 8	800 µl Washing Solution A
Cavity 3 + 9	800 µl Washing Solution B2
Cavity 4 + 10	800 µl Washing Solution B2
Cavity 5 + 11	empty
Cavity 6 + 12	100 µl – 200 µl Elution Buffer

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.

10.2 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.

   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

Store the DNA under adequate conditions.

We recommend storing the extracted DNA for longer use at −22°C to −18°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 Elution Buffer	Elute the DNA in a lower volume of Elution Buffer (min. 80 µl).
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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