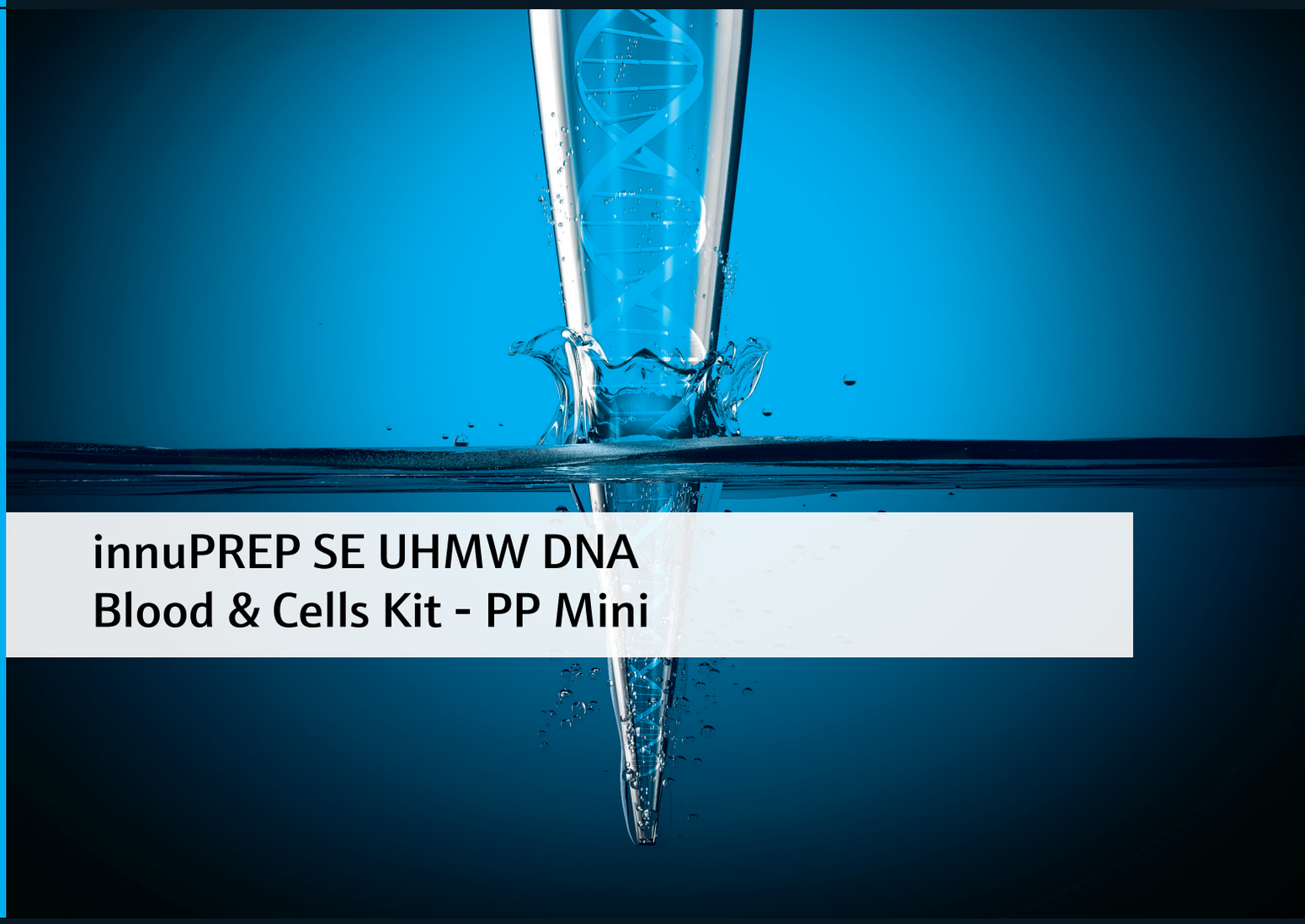
Instructions for Use
Life Science Kits & Assays



innuPREP SE UHMW DNA Blood & Cells Kit - PP Mini

Innuscreen GmbH

Order No.:

845-PSS-4016016	16 reactions
845-PSP-4016016	16 reactions
845-PSS-4016096	96 reactions
845-PSP-4016096	96 reactions

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Contents

1 Introduction

1.1 Intended use

The **innuPREP SE UHMW DNA Blood & Cells Kit – PP Mini** has been designed for automated isolation of ultra-high molecular weight (UHMW) from cultivated eukaryotic cells or peripheral blood mononuclear cells (PBMC) derived from fresh or frozen blood stabilized with EDTA, citrate or heparin based on a patented technology.

For blood samples the procedure starts with the lysis of erythrocytes and the subsequent pelleting of the PBMC's. After resuspension, the cells are transferred into the Deep Well Plate/Strip. For cultivated eukaryotic cells no preliminary steps are necessary. The extraction process is based on adsorption of the genomic DNA on so called Smart Modified Surfaces and it needs no magnetic particles for DNA binding. That means, the DNA binds directly on the surface of the modified Tip Combs. After washing, the genomic DNA is dissolved and is ready-to-use for subsequent downstream applications.

The whole extraction process just needs simple mixing up and down of the modified Tip Combs. The process is very fast and gives no limitation regarding the binding capacity. So, the kit is optimized to get a maximum of yield and quality.

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 and the kit“ p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before 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 might be used with 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** at 4 °C to 8 °C and **RNase A** at − 22 to -18 °C.

All other components of the **innuPREP SE UHMW DNA Blood & Cells Kit – PP Mini** 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 SE UHMW DNA Blood & Cells 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-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. 9). 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

The kit is for research use only!

6 Kit components

6.1 Components included in the kit

	Σ 16	Σ 96
REF	845-PSS-4016016 [a] 845-PSP-4016016 [b]	845-PSS-4016096 [a] 845-PSP-4016096 [b]
Ery Lysis Solution A (conc.)	2 x 11 ml	2 x 60 ml
Ery Lysis Solution B (conc.)	10 ml	60 ml
Lysis Solution CBV	5 ml	25 ml
Proteinase K	for 1 x 1.5 ml working solution	for 4 x 1.5 ml working solution
RNase A	2 x 60 µl	600 µl
Buffer H1	2 x 3 ml	25 ml
Buffer H2	1 ml	6 ml
Washing Solution MS (conc.)	9 ml	54 ml
Washing Solution ER	17 ml	85 ml
Elution Buffer	7 ml	50 ml
Modified Tip Comb [a]	4	24
Modified Tip Comb [b]	2	12
DW Strip [a]	16	96
KF96 DW Plate [b]	1	6
Manual	1	1

6.2 Components not included in the kit

- 96 %–99.8 % ethanol (molecular biology grade, undenatured)
- 70 % ethanol (molecular biology grade, undenatured)
- ddH_2O; ultrapure for dissolving Proteinase K and for diluting Ery Lysis Solution A (conc.) and Ery Lysis Solution B (conc.)

7 Product specifications

1. Starting material:

- Eukaryotic cells ($1 \times 10^6 - 1 \times 10^7$)
- Whole blood for collecting of PBMC's (1 – 3 ml; depends on amounts of PBMC's. Do not use more blood than correspond to 1×10^7 PBMC's)

2. Time for automated extraction:

- approx. 2.5 hours

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-PSS/PSP-4016016	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-PSS/PSP-4016096	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of ethanol to **Washing Solution MS (conc.)** and mix thoroughly. Always keep the bottles firmly closed!

845- PSS/PSP-4016016	Add 21 ml ethanol to 9 ml **Washing Solution MS**.
845- PSS/PSP-4016096	Add 126 ml ethanol to 54 ml **Washing Solution MS**.

- Use appropriate bottles and add the indicated volumes of **Ery Lysis Solution A (conc.)** to ddH_2O and mix thoroughly. Always keep the bottles firmly closed!

845- PSS/PSP-4016016	Add 11 ml **Ery Lysis Solution A** to 99 ml ddH_2O.
845- PSS/PSP-4016096	Add 60 ml **Ery Lysis Solution A** to 540 ml ddH_2O.

- Use appropriate bottles and add the indicated volumes of **Ery Lysis Solution B (conc.)** to ddH_2O and mix thoroughly. Always keep the bottles firmly closed!

845- PSS/PSP-4016016	Add 10 ml **Ery Lysis Solution B** to 90 ml ddH_2O.
845- PSS/PSP-4016096	Add 60 ml **Ery Lysis Solution B** to 540 ml ddH_2O.

9 Sample preparation

9.1 Sample preparation from 1-3 ml whole blood

1. Dispense Ery Lysis Solution A according to the volume of whole blood sample (see table below) into a 15 ml tube.

Whole blood volume	Volume of Ery Lysis Solution A
1.0 ml	5.0 ml
2.0 ml	8.0 ml
3.0 ml	10.0 ml

1. Add 1 – 3 ml whole blood into the prepared 15 ml tube and mix by inverting 6 times.
2. Incubate 5–10 minutes at room temperature. Invert at least once during incubation time.

NOTE
For fresh blood (collected within 1–6 h before starting the extraction) increase incubation time to 10 minutes to ensure complete lysis of red blood cells. Do not use more blood than correspond to 1 x 10^7 PBMC's!

3. Centrifuge for 3 minutes at 1,500 x g to pellet the PBMC.
4. **Carefully** discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet!

5. Add **5 ml Ery Lysis Solution B** to the PBMC pellet and vortex shortly or shake the tube vigorously to resuspend the cell pellet completely.
6. Centrifuge for 3 minutes at 1,500 x g to pellet the PBMC.
7. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet! Use a paper towel to remove residual liquid as much as possible!

8. Add **130 µl Ery Lysis Solution B** to the cell pellet and resuspend the pellet as much as possible by pipetting up and down. Adjust the pipet to 200 µl (not more).
9. Transfer max. 220 µl of resuspended cells into the first cavity of the DW plate.
10. Add **200 µl Lysis Solution CBV** and **30 µl Proteinase K** (for 3.0 ml blood sample use 50 µl Proteinase K) to the first cavity of the DW plate.

NOTE

Optionally add 5 µl RNase A (10mg/ml) to each sample.

Proceed with “Automated extraction using PurePrep Mini” on p.14.

9.2 Sample preparation from eucaryotic cells (1 x 10^6 – 1 x 10^7 cells)

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 3 minutes at 1,500 x g) and discard the supernatant as much as possible.
2. Add **130 µl Ery Lysis Solution B** to the cell pellet and resuspend the pellet as much as possible by pipetting up and down. Adjust the pipet to 200 µl (not more).
3. Transfer max. 220 µl of resuspended cells into the first cavity of the DW plate.
4. Add **200 µl Lysis Solution CBV** and **40 µl Proteinase K** to the first cavity of the DW plate.

NOTE

Optionally add 5 µl RNase A (10mg/ml) to each sample.

Proceed with “Automated extraction using PurePrep Mini” on p.14.

10 Automated extraction using 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

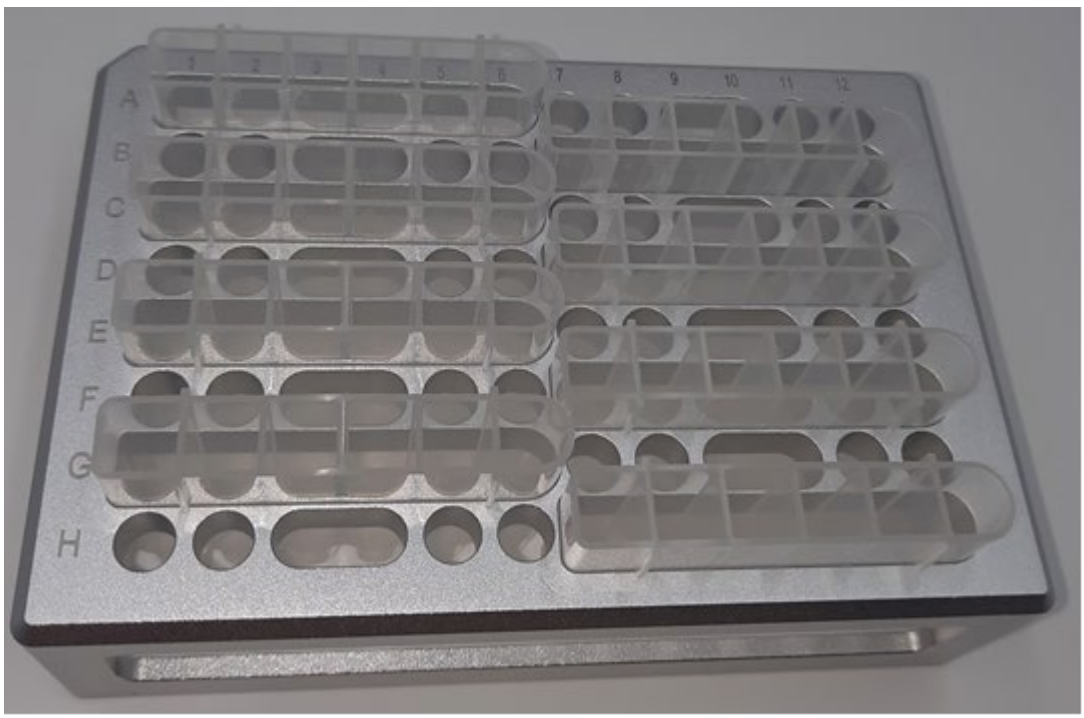

Fig. 2: Arrangement of the DW Strips in Tray

Cavity of DW Plate/Strip	Content
Cavity 1	Resuspended pellet + 200 µl Lysis Solution CBV + 30-50 µl Proteinase K
Cavity 2	800 µl Washing Solution MS
Cavity 3	800 µl Washing Solution MS
Cavity 4	800 µl 70% Ethanol
Cavity 5	800 µl Washing Solution ER
Cavity 6	400 µl Elution Buffer

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 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

   “PPMiniSE1“ and start the run.
2. After finishing the lysis the device stops.
3. Add 220 µl Buffer H1 + 40 µl Buffer H2 and press enter.
4. After finishing the extraction protocol, Cavity 6 and 12 contain the isolated UHMW DNA.
5. Transfer the DNA into a fresh 1.5 ml Tube using a wide-bore tip or a cut-off pipette tip.

IMPORTANT NOTE HIGH MOLECULAR WEIGHT DNA

The UHMW DNA might be very viscous. The dissolving step is crucial for successful extraction and for a maximum of yield. If the DNA content is too high, increase the amount of Elution Buffer.

UHMW gDNA needs time to relax. It is generally not recommended to work with freshly eluted DNA unless significant effort is made to ensure even DNA resuspension. Letting a sample relax overnight or for several days facilitates homogenization. If possible, it is recommended that UHMW DNA is extracted several days or a week prior to being needed for

downstream application.

If you do not need high molecular weight DNA you can shear the DNA e.g. by using ultrasound or by passing the eluate through a needle or a shredder spin filter unit.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Reduce amount of starting material.
Preparation without Buffer H1 and Buffer H2	Pay special attention that Buffer H1 and Buffer H2 was added to the lysed sample!
High viscosity extracted DNA / Inhomogeneous DNA sample	
Relax time to short	Refer to the note of UHMW DNA and let the DNA relax overnight at 2-8°C.
Degraded or sheared DNA	
Old material insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

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