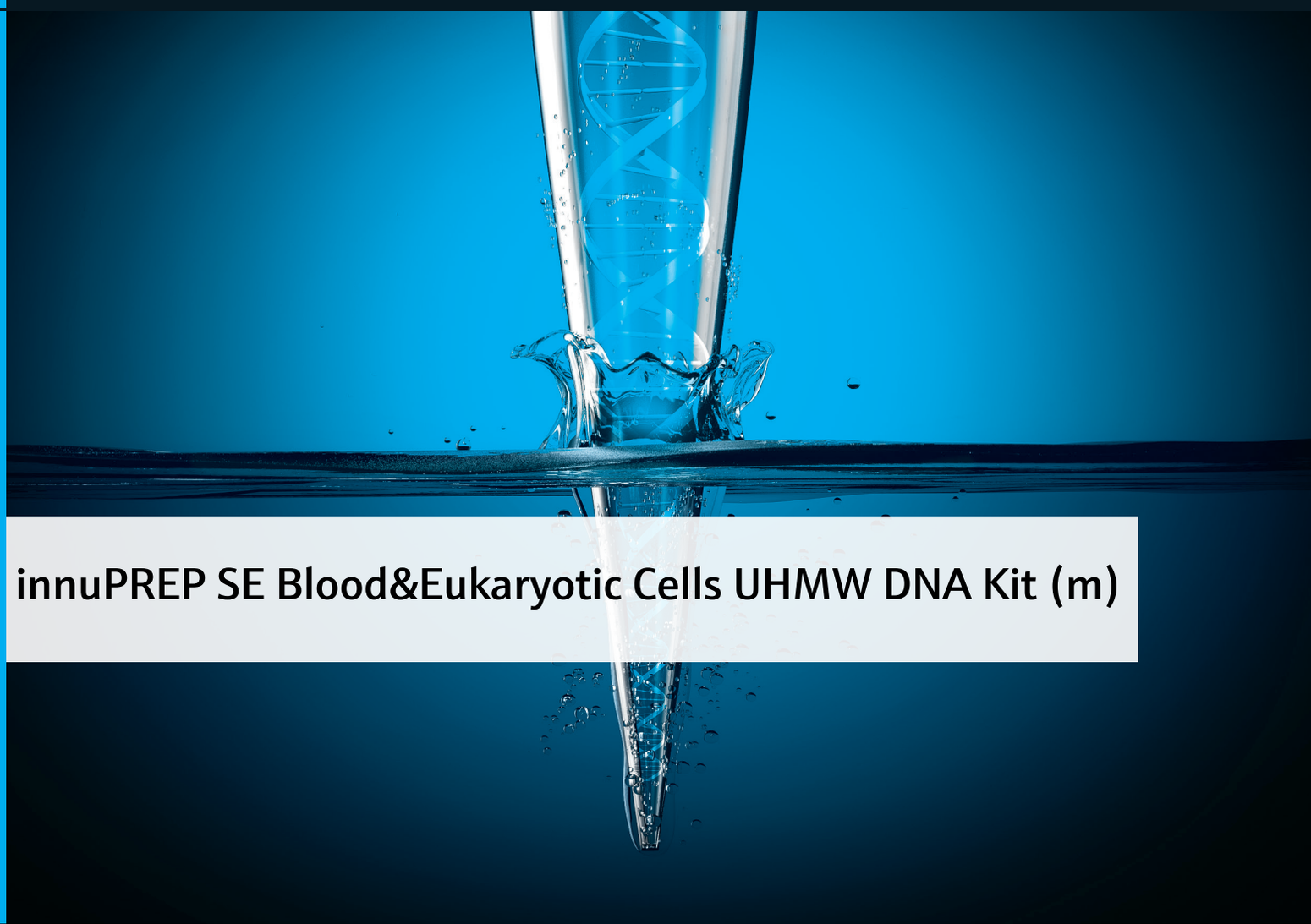
Instructions for Use
Life Science Kits & Assays



innuPREP SE Blood&Eukaryotic Cells UHMW DNA Kit (m)

Innuscreen GmbH

Order No.:
845-KS-8101010 10 reactions
845-KS-8101050 50 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 Contents

2 Introduction

2.1 Intended use

The **innuPREP SE Blood&Eukaryotic Cells UHMW DNA Kit (m)** has been designed for manual isolation of high molecular weight (HMW) and ultra-high molecular weight (UHMW)genomic DNA 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 addition of 1 x PBS, the cells are resuspended and transferred into the SmartExtraction Tubes followed by lysis. For cultivated eukaryotic cells no preliminary steps are necessary. The SE Tube contains a material with unique Smart Modified Surfaces which adsorbs the genomic DNA during the binding step. After washing steps, the nucleic acid is dissolved from the surface of the modified material and is now ready to use for downstream applications. The whole extraction process is simple to handle. The unique extraction chemistry in combination with Smart Modified Surfaces is optimized to get a maximum of yield and quality.

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.

2.2 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> tests.
30 °C 15 °C	**Storage conditions** Store at room temperature, unless otherwise specified.
	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 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.

3 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 is designed to be handled only 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 is to be used with potential infectious human 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 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.

4 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C and RNase A at -22 bis −18°C.

All other components of the **innuPREP SE Blood&Eukaryotic Cells UHMW DNA Kit (m)** 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 dissolve these precipitates by careful warming. Before every use make sure that all components have room temperature.

5 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 Blood&Eukaryotic Cells Kit (m)** kit 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.

6 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 equivalent regulations required in other countries.

All products sold by the 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!

7 Kit components

7.1 Included kit components

	Σ 10	Σ 50
REF	845-KS-8101010	845-KS-8101050
SE Tube	10	50
Ery Lysis Solution A (conc.)	25 ml	2 x 60 ml
Ery Lysis Solution B (conc.)	25 ml	2 x 60 ml
Lysis Solution CHV	3 ml	15 ml
Proteinase K	For 2 x 0.3 ml working solution	For 2 x 1.5 ml working solution
RNase A	60 µl	300 µl
Buffer H1	3 ml	15 ml
Buffer H2	1 ml	2 ml
Washing Solution MS (conc.)	6 ml	30 ml
Washing Solution ER	6 ml	17 ml
RNase-free Water	15 ml	2 x 30 ml
Manual	1	1

7.2 Components not included in the kit

- 1.5 ml and 2.0 ml tubes
- 80 % and 96–98.8 % ethanol (molecular biology grade, undenaturated)
- 2-Propanol (molecular biology grade)
- ddH_2O for dissolving **Proteinase K**
- appropriate flasks for preparing Ery Lysis Solutions
- PBS

7.3 Related Products

- magnetic rack for 1.5 and 2.0 ml tubes

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-KS-8100010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-8100050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- 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-KS-8100010	Add 25 ml Ery Lysis Solution A (conc.) to 225 ml ddH_2O.
845-KS-8100050	Add 60 ml Ery Lysis Solution A (conc.) 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 bottle firmly closed!

845-KS-8100010	Add 25 ml Ery Lysis Solution B (conc.) to 225 ml ddH_2O.
845-KS-8100050	Add 60 ml Ery Lysis Solution B (conc.) to 540 ml ddH_2O.

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

845-KS-8100010	Add 14 ml ethanol to 6 ml Washing Solution MS (conc.).
845-KS-8100050	Add 70 ml ethanol to 30 ml Washing Solution MS (conc.).

9 Product specifications

1. Starting material:
   - 0.5–10 ml whole blood (fresh or frozen) treated with EDTA, citrate or heparin.
   - Eukaryotic cells (1×10^5–1×10^7)
2. Typical yield:
   - Depending on amount and condition of PBMC.
   - Typical yields:

Whole blood volume	Typical yield
0.5 ml	5–15 µg
1.0 ml	15–30 µg
2.0 ml	40–70 µg
3.0 ml	50–90 µg
10 ml	> 300 µg

NOTE
Yield of isolated DNA is affected by amount and condition of PBMC used. The condition of PBMC depends on storage conditions as well as constitution of the donor. It has to be considered that a medical attendance of the donor may lower the yield of isolated DNA. This kit requires intact cells and may not work satisfying in case of damaged cells in starting material!

10 Lysis of erythrocytes, pelleting of PBMC and resuspension

10.1 Isolation from 0.5–3 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
0.5–1.0 ml	3.0 ml
2.0 ml	5.0 ml
3.0 ml	8.0 ml

2. Add **0.5–1 ml**, **2 ml** or **3 ml whole blood** into the prepared 15 ml tube and mix by inverting 6 times.
3. 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 20 minutes to ensure complete lysis of red blood cells.

4. Centrifuge for 5 minutes at 2,500 x g to pellet the PBMC.
5. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet!

6. 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.
7. Centrifuge for 5 minutes at 2,500 x g to pellet the PBMC.
8. 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!

9. Add **130 µl PBS** to the cell pellet and resuspend the pellet as much as possible by intensive pipetting up and down.
1. Transfer **max. 220 µl** of **resuspended PBMC** into a 2.0 ml Tube.
2. Add **200 µl Lysis Solution CHV** and **30 µl Proteinase K** (for > 3.0 ml blood sample use 50 µl Proteinase K). Vortex shortly and incubate the 2.0 ml Tube at 55 °C for 20 minutes in a thermal shaker continuously shaking with 1,000 rpm (for > 3.0 ml blood incubate for 30 minutes).
10. Proceed with "SmartExtraction protocol" on p. 13.

10.2 Isolation from 4–10 ml whole blood

1. Dispense **20 ml Ery Lysis Solution A** into a 50 ml tube.
2. Add **4–10 ml whole blood** into the prepared 50 ml tube and mix by inverting 10 times.
3. Incubate 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 20 minutes to ensure complete lysis of red blood cells.

4. Centrifuge for 5 minutes at 2,500 x g to pellet the PBMC.
5. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet!

6. Add **20 ml Ery Lysis Solution B** to the PBMC pellet and vortex shortly or shake the tube vigorously to resuspend the cell pellet completely.

7. Centrifuge for 5 minutes at 2,500 x g to pellet the PBMC.
8. 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!

9. Add **130 µl PBS** to the cell pellet and resuspend the pellet as much as possible by intensive pipetting up and down.
10. Transfer **max. 220 µl** of **resuspended PBMC** into a 2.0 ml Tube.
11. Add **200 µl Lysis Solution CHV** and **30 µl Proteinase K** (for > 3.0 ml blood sample use 50 µl Proteinase K). **Vortex shortly and incubate the 2.0 ml** Tube **at 55 °C for 20 minutes in a thermal shaker continuously shaking with 1,000 rpm** (for > 3.0 ml blood incubate for 30 minutes).
12. Proceed with “SmartExtraction protocol” on p. 13.

10.3 Isolation from eucaryotic cells

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 5 minutes at 2,500 x g) and discard the supernatant.
2. Add **100 µl PBS** to the cell pellet and resuspend the pellet.
3. Add **280 µl Lysis Solution CHV** and **30 µl Proteinase K**. Vortex briefly and incubate at 55 °C for 20 minutes in a thermal shaker under continuously shaking with 1,000 rpm.
4. Transfer the lysate to the **SE Tube**.
5. Proceed with “SmartExtraction protocol” on p. 13.

RECOMMENDED
To remove RNA from the sample (optional) add 5 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 10 minutes at room temperature. Be sure, that the RNase A is free of DNase-activity.

11 SmartExtraction protocol

NOTE
If not otherwise stated, we recommend using a magnetic rack to retain the SE Macro Beads in the SE Tubes and remove supernatants by pouring. Using pipets to aspirate the liquid phase might result in a loss of DNA.

11.1 Binding DNA to SE Macro Beads

1. After lysis add **220 µl Buffer H1** and 2**0 µl Buffer H2** to the sample in the SE Tube.
2. Carefully invert the SE Tube 30 times (invert 180°/per cycle 3 seconds). Leave SE tube for 2 min.

NOTE
Sample is very viscous at the beginning - viscosity is completely gone in the end.

3. Place the SE Tube into a magnetic rack for separation of the SE Macro Beads. Leave SE tube for 2 min. Carefully pour off the supernatant.

11.2 Washing and removing of alcohol

1. Add **800 µl Washing Solution MS** and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack three times. Carefully pour off the Washing Solution MS (do not remove the SE Tube from the magnetic rack).
2. Add **800 µl Washing Solution MS** and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack three times. Carefully pour off the Washing Solution MS (do not remove the SE Tube from the magnetic rack).
3. Add **800 µl of 70 % ethanol** and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack three times. Carefully pour off the ethanol (do not remove the SE Tube from the magnetic rack).

4. Add **500 µl Washing Solution ER** and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack three times. Carefully pour off the Washing Solution ER (do not remove the SE Tube from the magnetic rack).
5. Let the SE Tube rest in the magnetic rack and wait some seconds. Carefully remove as much as possible of the residual liquid using a pipet (also remove ethanol which can be insight the cap).

11.3 Elution of DNA from SE Macro Beads

1. Add **200 µl RNase free Water** to the SE Tube. Incubate the SE Tube in a thermal shaker for 30 minutes at 37 °C shaking with 300 rpm. For further analysis please refer to the recommendation of the important note for HMW DNA!!! Let the DNA relax with the beads, don't transfer before relaxing. Let stay the tube with beads over night at 4-8°C
2. Place the SE Tube into the magnetic rack and transfer the DNA into a new tube using a wide bore pipet tip.

IMPORTANT NOTE HIGH MOLECULAR WEIGHT DNA
The HMW 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 and/or prolong the elution step. HMW 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 HMW 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.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material.
Inappropriately treated starting material	Avoid freezing and thawing of starting material.
Incomplete elution	Prolong the elution time.
High viscosity extracted DNA	
Insufficient amount of Elution Buffer	Elute the DNA with a higher volume of Elution Buffer.
Degraded or sheared DNA	
Old material	Old material often contains degraded DNA.
Pipetting steps performed too rigorous	Pipet more carefully and/or use wide bore pipette tips.

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