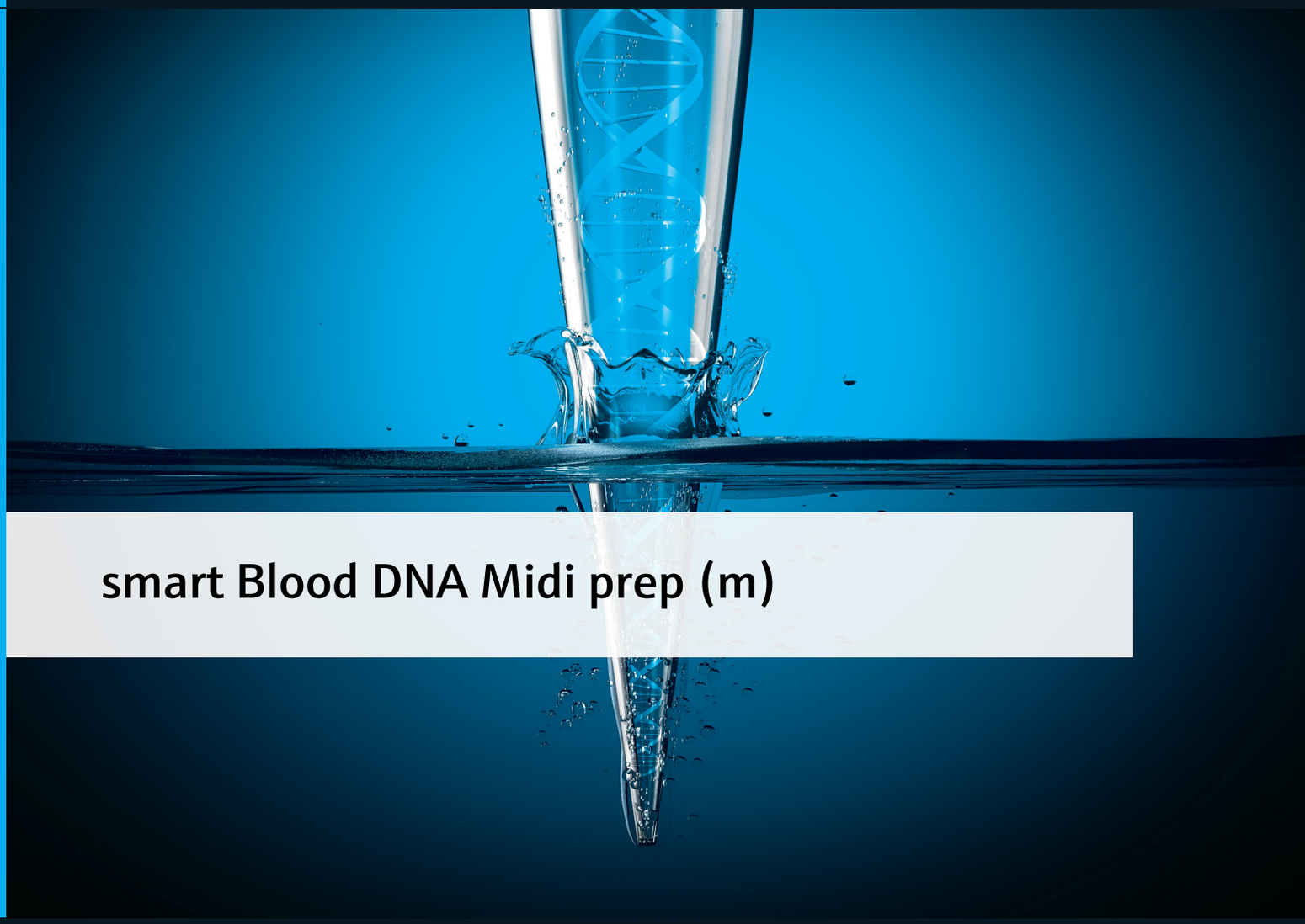
Instructions for Use
Life Science Kits & Assays



smart Blood DNA Midi prep (m)

Innuscreen GmbH

Order No.:

845-KS-8100010 10 reactions
845-KS-8100050 50 reactions

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1 Introduction

1.1 Intended use

The smart Blood DNA Midi prep (m) has been designed for manual isolation of high molecular weight genomic DNA from peripheral blood mononuclear cells (PBMC) derived from fresh or frozen blood stabilized with EDTA, citrate or heparin. The kit utilizes the new SmartExtraction technology invented by IST Innuscreen GmbH (patent pending).

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. 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.

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> 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.

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 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.

3 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival store lyophilized and dissolved **Proteinase K** at 4 ℃ to 8 ℃ .

All other components of the smart Blood DNA Midi prep (m) kit should be stored dry at room temperature (15 ℃ to 30 ℃). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

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 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 smart Blood DNA Midi prep (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.

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 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
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50
REF	845-KS-8100010	845-KS-8100050
SE Tube	10	50
1 x PBS	2 ml	10 ml
Ery Lysis Solution A (conc.)	25 ml	2 x 60 ml
Ery Lysis Solution B (conc.)	25 ml	2 x 60 ml
Lysis Solution CBV	5 ml	15 ml
Proteinase K	For 2 x 0.3 ml working solution	For 2 x 1.5 ml working solution
Binding Optimizer	1 ml	3 x 1 ml
Washing Solution LS (conc.)	2 ml	12 ml
Elution Buffer	15 ml	2 x 30 ml
Manual	1	1

6.2 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

6.3 Related Products

- magnetic rack for 1.5 and 2.0 ml tubes (IST Innuscreen GmbH, 845-MR-0600001)

7 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.

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

845-KS-8100010	Add 8 ml ethanol to 2 ml Washing Solution LS (conc.).
845-KS-8100050	Add 48 ml ethanol to 12 ml Washing Solution LS (conc.).

- Use appropriate bottles and add the indicated volumes of **Ery Lysis Solution A (conc.) and** ddH_2O and mix thoroughly. Always keep the bottle 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 B2 (conc.) and** ddH_2O and mix thoroughly. Always keep the bottle 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.

8 Product specifications

1. Starting material:
   - 0.5–10 ml whole blood (fresh or frozen) treated with EDTA, citrate or heparin.
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!

9 Lysis of erythrocytes, pelleting of PBMC and resuspension

9.1 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.
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 **120 µl PBS** to the cell pellet and resuspend the pellet as much as possible by intensive pipetting up and down.
10. Proceed with "SmartExtraction protocol" on p. 13.

9.2 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.
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 1 x PBS** to the cell pellet and resuspend the pellet as much as possible by intensive pipetting up and down.

IMPORTANT NOTE

If the amount of PBMC is extremely high, the resuspension of pellet using 130 µl of 1 x PBS might not be sufficient. In this case, add additional 200 µl of 1 x PBS and resuspend the pellet. Then split the sample in two equal parts of 220 µl and do two separate extractions and pool the DNA finally.

10. Proceed with “SmartExtraction protocol” on p. 13.

10 SmartExtraction protocol

NOTE
If not otherwise stated, we recommend to use 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.

10.1 Lysis of PBMC

1. Transfer **max. 220 µl** of **resuspended PBMC** into the **SE Tube**.
2. Add **200 µl Lysis Solution CBV** and **50 µl Proteinase K**. Vortex shortly and incubate the **SE Tube** at 55 °C for 30 minutes in a thermal shaker continuously shaking with 1,200 rpm.

10.2 Binding DNA to SE Macro Beads

1. After lysis add **40 µl Binding Optimizer** and **350 µl 2-Propanol** to the sample in the SE Tube.
2. Place the SE Tube into a thermal shaker and incubate for 3 minutes at room temperature shaking with 1,400–1,800 rpm.
3. Place the SE Tube into a magnetic rack for separation of the SE Macro Beads. Discard the supernatant.

10.3 Washing and removing of alcohol

1. Add 800 µl of Washing Solution LS and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack five times. Discard the Washing Solution LS (do not remove the SE Tube from the magnetic rack).
2. Add 800 µl of 80 % ethanol and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack five times. Discard the ethanol (do not remove the SE Tube from the magnetic rack).
3. Add 800 µl of 80 % ethanol and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack five times. Discard the ethanol (do not remove the SE Tube from the magnetic rack).

4. Let the SE Tube rest in the magnetic rack and wait some seconds. Carefully remove as much as possible of the ethanol using a pipet (remove also ethanol which can be insight the cap).
5. Place the SE Tube with opened cap in a thermal shaker and incubate for 15 minutes at 65 °C shaking with 400 rpm to remove the ethanol completely. If the ethanol is not completely removed prolong the incubation time.

10.4 Elution of DNA from SE Macro Beads

1. Add 200 µl–1,000 µl of Elution Buffer to the SE Tube. The amount depends on starting amount of sample and expected yield. Incubate the SE Tube in a thermal shaker for 15 minutes at 65 °C shaking with1,000 rpm.

   If the DNA is not completely dissolved (DNA is visible as a clump or a strand) a prolongation of the elution step is strongly recommended.
2. Place the SE Tube into the magnetic rack and transfer the DNA into a new tube using a pipet.

IMPORTANT NOTE
The extracted DNA is of high molecular weight (200 kb–500 kb). Therefore, the 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 prolong the elution step.
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.

11 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 incubation time with Elution Buffer.
Preparation without Binding Optimizer	It is important to add the Binding Optimizer to the lysed sample as described. Binding Optimizer need to be added after lysis of sample is finished!
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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