Instructions for Use
Life Science Kits & Assays

smart Blood DNA Midi prep (m)

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-8100010 10 reactions
845-KS-8100050 50 reactions

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Contents

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 Analytik Jena (patent pending).

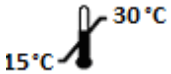
The procedure starts with the lysis of the starting material. After lysis the sample is transferred into a SmartExtraction Tube (SE Tube). The SE Tube contains a material with unique Smart Modified Surfaces which adsorbs the genomic DNA. After washing steps, the nucleic acid is dissolved from the surface 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 prior to 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

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains sufficient reagents for <N> tests.
	**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 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 prior 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, please ask for the material safety data sheets (MSDS's).

3 Storage conditions

Store lyophilized **Proteinase K** at 4 °C to 8 °C! Divide dissolved **Proteinase K** into aliquots and store at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!

All other components of the smart Blood DNA Midi prep (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.

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. For further information see chapter "Kit components" (→ p. 7).

4 Function testing and technical assistance

The Analytik Jena AG 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 smart Blood DNA Midi prep (m) kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

5 Product use and warranty

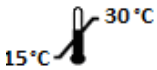
The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ "Intended use") (→ "Product specifications"). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols than those described below. Analytik Jena AG 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 Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
For research use only!

6 Kit components

IMPORTANT
Store lyophilized **Proteinase K** at 4 °C to 8 °C! Aliquot dissolved **Proteinase K** and store at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!



STORAGE CONDITIONS
All other components are stored at room temperature.

	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 (final volume 250 ml)	2 x 60 ml (final volume 2 x 600 ml)
Ery Lysis Solution B (conc.)	25 ml (final volume 250 ml)	2 x 60 ml (final volume 2 x 600 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 (final volume 10 ml)	12 ml (final volume 60 ml)
Elution Buffer	15 ml	2 x 30 ml
Manual	1	1

Initial steps	**Washing Solution LS (conc.)** Add 8 ml of 96-99.8 % ethanol to the bottle Washing Solution LS mix thoroughly and keep the bottle always firmly closed!	**Washing Solution LS (conc.)** Add 48 ml of 96-99.8 % ethanol to the bottle Washing Solution LS mix thoroughly and keep the bottle always firmly closed!
	Ery Lysis Buffer A (conc): Use an appropriate bottle and add 225 ml of ddH_2O and 25 ml Ery Lysis Solution A (conc.) and mix thoroughly. Keep the bottle always firmly closed.	**Ery Lysis Buffer A (conc.):** Use appropriate bottles and add 540 ml and 60 ml Ery Lysis Solution A (conc.) and mix thoroughly. Keep the bottle always firmly closed.
	Ery Lysis Buffer B (conc.): Use an appropriate bottle and add 225 ml of ddH_2O and 25 ml Ery Lysis Solution B (conc.) and mix thoroughly. Keep the bottle always firmly closed.	**Ery Lysis Buffer B (conc.):** Use appropriate bottles and add 540 ml of ddH_2O and 60 ml Ery Lysis Solution B (conc.) and mix thoroughly. Keep the bottle always firmly closed.
	Proteinase K: Dissolve Proteinase K by addition of 0.3 ml of ddH_2O, mix thoroughly and store as described!	**Proteinase K:** Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described!

6.1 Components not included in the kit

- 1.5 ml and 2.0 ml tubes
- 96–98.8 % ethanol (molecular biology grade, undenaturated)
- 80 % ethanol (molecular biology grade, undenaturated)
- 2-Propanol (molecular biology grade)
- ddH_2O
- magnetic rack (Analytik Jena AG, 845-MR-0600001)
- appropriate flasks for preparing Ery Lysis Solutions

6.2 Recommended steps before starting

- Ensure that the **Washing Solution LS**, **Ery Lysis Solution A**, **Ery Lysis Solution B** and the **Proteinase K** have been prepared according to the instruction.
- Avoid repeated freezing and thawing of starting material.

7 GHS Classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Ery Lysis Solution A (conc.)	Polyethylene glycol octylphenol ether 10–≤25 %	 Danger	315, 318, 412	101, 102, 103, 280, 273, 305+351+338, 310, 332+313, 501
Binding Optimizer	Acetic acid 10–≤25 %	 Warning	315, 319	101, 102, 103, 280, 305+351+338, 362, 302+352, 403+233, 501
Proteinase K	Proteinase, Tritirachium album serine	 Danger	315, 319, 334, 317, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501

7.1 Hazard phrases

315 Causes skin irritation.

317 May cause an allergic skin reaction.

318 Causes serious eye damage.

319 Causes serious eye irritation.

334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

335 May cause respiratory irritation.

412 Harmful to aquatic life with long lasting effects.

7.2 Precaution phrases

101	If medical advice is needed, have product container or label at hand.
102	Keep out of reach of children.
103	Read label before use.
261	Avoid breathing dust/fume/gas/mist/vapors/spray.
273	Avoid release to the environment.
280	Wear protective gloves/protective clothing/ eye protection/face protection.
310	Immediately call a POISON CENTER/doctor.
362	Take off contaminated clothing.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
302+352	IF ON SKIN: Wash with plenty of water.
332+313	If skin irritation occurs: Get medical advice/attention.
342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
403+233	Store in a well-ventilated place. Keep container tightly closed.
305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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.

Proceed with “SmartExtraction protocol” on p. 18.

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.

Proceed with “SmartExtraction protocol” on p. 18.

10 SmartExtraction protocol

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 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 stay the **SE Tube** in the magnetic rack and wait some seconds and 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.

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 to rigorous	Pipet more carefully and/or use wide bore pipette tips.

12 Related Products

Name	Amount	Order No.
Additional products for nucleic acid purification		
innuPREP Bacteria Lysis Booster	50 rxn	845-KA-1000050
innuPREP Proteinase K	6 mg	845-CH-0010006
	30 mg	845-CH-0010030
Kits for Nucleic acid purification		
smart DNA prep (m)	10 rxn	845-KS-8000010
	50 rxn	845-KS-8000050
Equipment		
Magnetrack 6fold	For up to six samples	845-MR-0600001
TS1 ThermoShaker, 100–240 V	EU plug	846-051-500
	US plug	846-051-590
Block Module 24 x 2,0 ml tubes for TS1		846-051-516

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Subject to changes in design and scope of delivery as well as further technical development!