Instructions for Use
Life Science Kits & Assays

innuPREP Blood DNA Mini Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-1120010 10 reactions
845-KS-1120050 50 reactions
845-KS-1120250 250 reactions

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1 Introduction

1.1 Intended use

The innuPREP Blood DNA Mini Kit has been designed as a very efficient tool for fast isolation of genomic DNA from whole blood samples for subsequent in vitro diagnostic purposes. The kit can be used for isolation of genomic DNA from fresh or frozen blood; stabilized with EDTA or citrate, from common blood collection systems. Sample volumes from 200 µl up to 400 µl can be processed. If smaller volumes of blood are used, apply sterile PBS up to 200 µl final sample volume.

The extraction procedure is based on a new patented chemistry and combines lysis of blood sample with subsequent binding of nucleic acids onto the surface of a Spin Filter membrane. After several washing steps the nucleic acids are eluted from the membrane by using Elution Buffer. Extraction chemistry and extraction protocol are optimized to get maximum of yield.

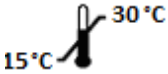
The innuPREP Blood DNA Mini Kit is not for use with cell-free body fluids such as cerebrospinal fluid, serum, plasma or urine, tissue or stool samples. The kit performance has not been evaluated with buffy coat, cultured or isolated cells, swabs, dried blood spots and viral DNA. The kit is also not specified for the isolation and purification of fungal, bacterial or parasite nucleic acids.

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. Diagnostic results generated using the extraction procedure in conjunction with diagnostic tests should be interpreted with regard to other clinical or laboratory results. To reduce irregularities in diagnostic results, internal controls for downstream applications should be used.

The innuPREP Blood DNA Mini Kit does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream in vitro diagnostic assay.

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> reactions.
	**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 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

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.

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 could be used with potential 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.

3 Storage conditions

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

All other components of the innuPREP Blood DNA Mini 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.

For further information see chapter “Kit components” (→ p. 6).

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 innuPREP Blood DNA Mini 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

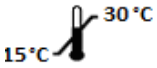
The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use” p. 2) (→ “Product specifications” p. 9). Since the performance characteristics of our kits have not been validated for any specific application. 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 equivalents 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
The kit is for research use only!

6 Kit components

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



STORAGE CONDITIONS
All other components are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	845-KS-1120010	845-KS-1120050	845-KS-1120250
Lysis Solution SLS	5 ml	25 ml	120 ml
Binding Solution BL	8 ml	40 ml	200 ml
Proteinase K	for 1 x 0.3 ml working solution	for 2 x 1.5 ml working solution	for 6 x 1.5 ml working solution
Washing Solution C	5 ml	25 ml	120 ml
Washing Solution BS (conc.)	2 ml	8 ml	2 x 18 ml
Elution Buffer	2 x 2 ml	6 x 2 ml	3 x 25 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	50	5 x 50	25 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

	Σ 10	Σ 50	Σ 250
Initial steps	**Washing Solution BS** Add 18 ml of 96-99.8 % ethanol to the bottle Washing Solution BS, mix thoroughly and keep the bottle always firmly closed!	**Washing Solution BS** Add 72 ml of 96-99.8 % ethanol to the bottle Washing Solution BS, mix thoroughly and keep the bottle always firmly closed!	**Washing Solution BS** Add 162 ml of 96-99.8 % ethanol to each bottle Washing Solution BS, mix thoroughly and keep the bottle always firmly closed!
	Proteinase K Dissolve by addition of 0.3 ml of ddH_2O, mix thoroughly and store as described above.	**Proteinase K** Dissolve by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described above.	**Proteinase K** Dissolve by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described above.

Components not included in the kit

- RNase A (10 mg/ml); optional
- 1.5 ml tubes
- 2.0 ml tubes; optional
- 96–99.8 % ethanol
- ddH_2O
- PBS

NOTE

Use only absolute/pure ethanol, NO methylated or denatured alcohol!

7 Product specifications

1. Starting material:
   - Whole blood (200 µl or up to 400 µl)
   - Fresh or frozen material
   - Stabilizers: EDTA or citrate

2. Time for isolation:
   - Approximately 24 minutes including lysis step

3. Typical yield:
   - Depends on sample (amount of nucleated cells) and amount of starting material
   - Up to > 30 µg gDNA

4. Typical ration A_{260}:A_{280}:
   - 1.7–2.0

8 GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Lysis Solution SLS	Ammonium chloride 10–<50 % Cetrimonium bromide 1–<2.5 %	 *Danger*	319, 400	101,102,103, 280,273,264, 305+351+338, 501
Binding Solution BL	Propan-2-ol 50–100 %	 *Danger*	225,319,336	101,102,103,210,261,303+361+353,305+351+338,405,501
Washing Solution C	Propan-2-ol 25–50 %	 *Danger*	225,319,336	101, 102, 103, 210,261,303+361+353, 305+351+338, 405, 501
Proteinase K	Proteinase, engyodontium album	 *Danger*	315, 319, 334, 317, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501

8.1 Hazard phrases

225	Highly flammable liquid and vapour.
315	Causes skin irritation.
317	May cause an allergic skin reaction.
319	Causes serious eye irritation.
334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
335	May cause respiratory irritation.
336	May cause drowsiness or dizziness.
400	Very toxic to aquatic life.

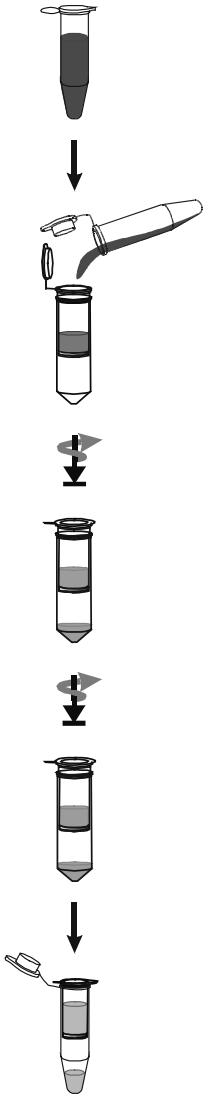
8.2 Precaution phrases

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.
210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
261	Avoid breathing dust/fume/gas/mist/vapours/spray.
264	Wash thoroughly after handling.
273	Avoid release to the environment.
280	Wear protective gloves/protective clothing/eye protection/face protection.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
305+351+ 338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

9 Recommended steps before starting

- Heat thermal mixer or water bath at 60 °C.
- Ensure that the **Washing Solution BS** and **Proteinase K** have been prepared according to the instruction (→ "Kit components" p. 6).
- Preheat the **Elution Buffer** at 60 °C.
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

10 General procedure for DNA extraction



Lysis of starting material

Binding of DNA on Spin Filter

Washing of the bound DNA

Elution of DNA

11 Protocol 1: DNA isolation from 200 µl whole blood samples

IMPORTANT

Pre-fill the needed amount of **Elution Buffer** into a 2.0 ml reaction tube and incubate the **Elution Buffer** at 60 °C until the elution step.

If the sample volume is less than 200 µl, add the appropriate volume of PBS.

1. Pipette **200 µl** of **whole blood sample** into a 1.5 ml reaction tube.
2. Add **200 µl Lysis Solution SLS** and **20 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 60 °C for 10 minutes.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample.

Alternatively, vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.

NOTE

The kit co-purify DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution BL**, vortex shortly and incubate for 5 minutes at room temperature.

3. Optional: centrifuge the 1.5 ml reaction tube for 10 seconds to remove condensate from the lid of the reaction tube.

4. Open the 1.5 ml reaction tube and add **350 µl Binding Solution BL** to the lysed sample. Mix carefully by pipetting up and down several times (3 – 4 times), apply the sample using the pipette to a Spin Filter located in a Receiver Tube and close the cap of the Spin Filter.

IMPORTANT NOTE

Don't vortex the sample at this step!

It is important that the sample and the Binding Solution BL are mixed by pipetting up and down several times. Vortexing will lead to reduced yield of DNA.

5. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Open the Spin Filter and add **400 µl Washing Solution C**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
7. Open the Spin Filter and add **600 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter into a new Receiver Tube.
8. Open the Spin Filter and add **600 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
9. Centrifuge at max. speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.

10. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **200 µl Elution Buffer** (pre-warmed at 60 °C). Incubate at room temperature for 2 minutes.
11. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of pre-warmed Elution Buffer (e.g. 100 µl + 100 µl) might increase the yield of extracted gDNA.

NOTE

The DNA can be eluted with a lower or a higher volume of **Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

12 Protocol 2: DNA isolation from 400 µl whole blood samples

IMPORTANT
Pre-fill the needed amount of Elution Buffer into a 2.0 ml reaction tube and incubate the Elution Buffer at 60 °C until the elution step.

1. Pipette **400 µl** of **whole blood sample** into a 2.0 ml reaction tube.
2. Add **400 µl Lysis Solution SLS** and **30 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 60 °C for 10 minutes.

NOTE
We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.

NOTE
The kit co-purify DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution BL**, vortex shortly and incubate for 5 minutes at room temperature.

3. Optional: centrifuge the 1.5 ml reaction tube for 10 seconds to remove condensate from the lid of the reaction tube.

4. Open the 1.5 ml reaction tube and add **700 µl Binding Solution BL** to the lysed sample. Mix carefully by pipetting up and down several times (3 – 4 times), apply **750 µl** of the sample using the pipette to a Spin Filter located in a Receiver Tube and close the cap of the Spin Filter.

IMPORTANT NOTE

Don't vortex the sample at this step!

It is important that the sample and the Binding Solution BL are mixed by pipetting up and down several times. Vortexing will lead to reduced yield of DNA.

5. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Apply the residual sample to the Spin Filter. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

7. Open the Spin Filter and add **400 µl Washing Solution C**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

8. Open the Spin Filter and add **600 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter into a new Receiver Tube.

9. Open the Spin Filter and add **600 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

10. Centrifuge at max. speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.

11. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **200 µl Elution Buffer** (pre-warmed at 60 °C). Incubate at room temperature for 2 minutes.

12. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of pre-warmed Elution Buffer (e.g. 100 µl + 100 µl) might increase the yield of extracted gDNA.

NOTE

The DNA can be eluted with a lower or a higher volume of **Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homogenization	Increase lysis time. Increase centrifugation speed. Reduce amount of starting material.
Little or no DNA eluted	
Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer to 5 minutes or repeat elution step once again. Take a higher volume of Elution Buffer.
Insufficient mixing with Binding Solution BL	Mix sample with Binding Solution BL by pipetting up and down several times prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much Elution Buffer	Elute the DNA with lower volume of Elution Buffer.
Degraded or sheared DNA	
Incorrect storage of starting material	Ensure that the starting material is frozen immediately in liquid N_2 or in minimum at 20° C and is stored continuously at -80° C! Avoid thawing of the material.
Old material insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	
RNA contaminations of extracted DNA	Perform RNase digestion

14 Related products

Name	Amount	Order No.
Products for PCR & Gel Electrophoresis		
innuPREP Gel Extraction Kit	10 rxn	845-KS-5030010
	50 rxn	845-KS-5030050
	250 rxn	845-KS-5030250
innuPREP PCRpure Kit	10 rxn	845-KS-5010010
	50 rxn	845-KS-5010050
	250 rxn	845-KS-5010250
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
50x inNucleotide Mix (12.5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
innuDRY Standard PCR Master Mix	100 rxn	845-AS-2100100
	200 rxn	845-AS-2100200
innuMIX Standard PCR MasterMix	100 rxn	845-AS-1700100
	200 rxn	845-AS-1700200
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500

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