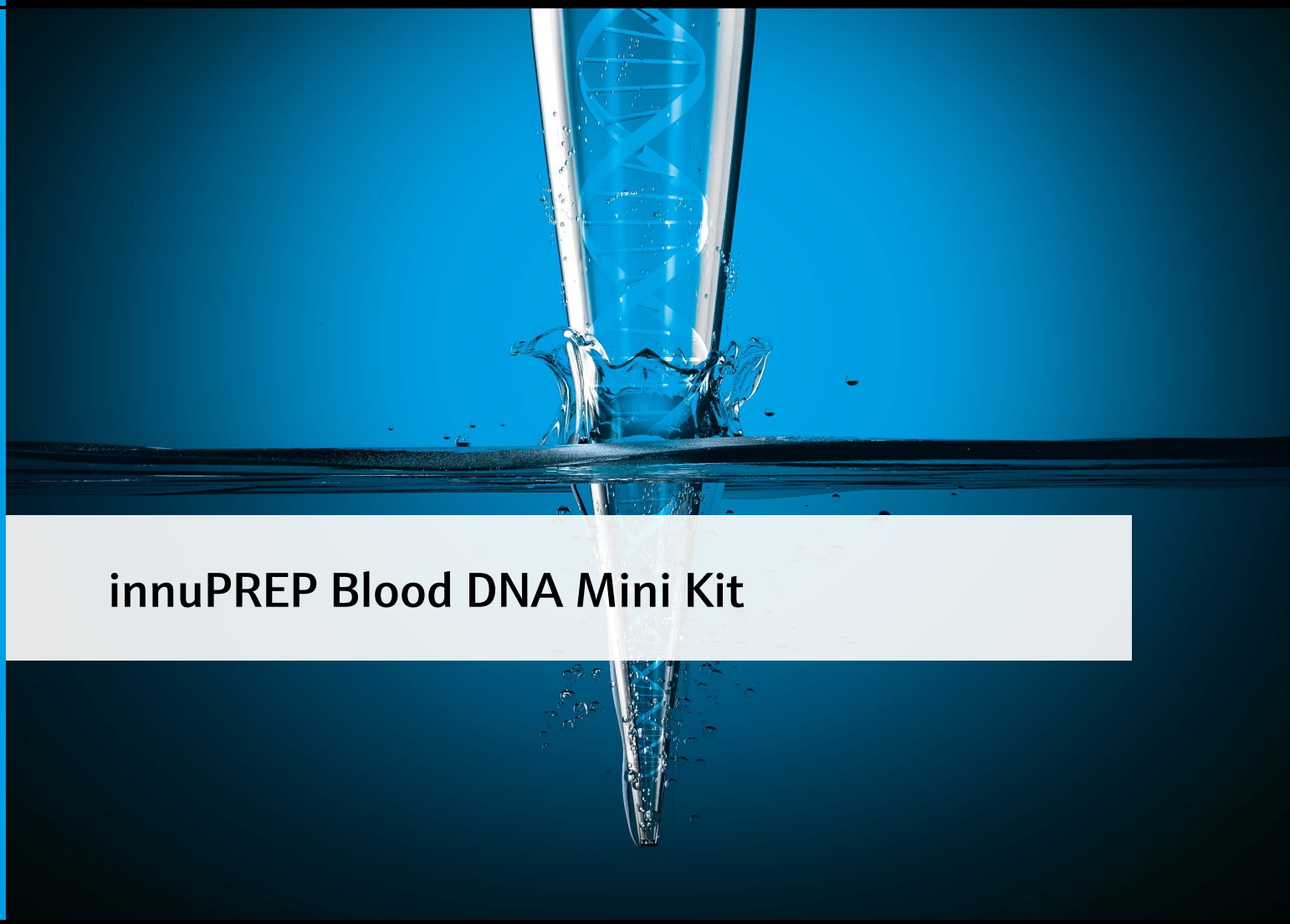
Instructions for Use
Life Science Kits & Assays



innuPREP Blood DNA Mini Kit

Innuscreen
innovative
Sensor
Technology

Order No.:

845-KS-1120010 10 reactions
845-KS-1120050 50 reactions
845-KS-1120250 250 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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Contents

1 Introduction 2
1.1 Intended use 2
1.2 Notes on the use of this manual and the kit 3
2 Safety precautions 4
3 Storage conditions 5
4 Functional testing and technical assistance 5
5 Product use and warranty 6
6 Kit components 7
6.1 Included kit components 7
6.2 Components not included in the kit 7
7 Product specifications 8
8 Initial steps before starting 9
9 Protocols for DNA isolation from blood samples 10
9.1 Protocol 1: Isolation from 200 µl samples 10
9.2 Protocol 2: Isolation from 400 µl samples 13
10 Troubleshooting 16

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 regarding 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.

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" 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 personal 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 could be used with potentially 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

The kit is shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

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.

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 kit was 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 **innuPREP Blood DNA Mini 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. 8). Since the performance characteristics of our kits have not been validated for any specific application. 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 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 Components included in the kit

	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	12 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

6.2 Components not included in the kit

- 1.5 ml tubes
- 96–99.8 % ethanol (molecular biology grade, undenatured)
- ddH_2O for dissolving Proteinase K
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- RNase A (10 mg/ml); optional
- 2.0 ml tubes; optional

7 Product specifications

1. Starting material:

- Fresh or frozen whole blood samples
- Stabilizers: EDTA or citrate
- 200 µl sample volume
- 400 µl sample volume

NOTE
Avoid freezing and thawing of starting material.

2. Time for isolation:

- Approximately 24 minutes including lysis step

3. Typical yield:

- Depends on sample quality
- Depends on amount of mononuclear blood cells
- Up to > 30 µg gDNA

4. Typical ration A_{260}:A_{280}:

- 1.7–2.0

8 Initial steps before starting

- Heat thermal mixer or water bath at 60 °C.
- Add the indicated amount of ddH_2O to **Proteinase K**, mix thoroughly and keep the bottle firmly closed.

845-KS-1120010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-1120050 845-KS-1120250	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of absolute ethanol to **Washing Solution BS (conc.)**, mix thoroughly and keep the bottle firmly closed.

845-KS-1120010	Add 18 ml ethanol to 2 ml Washing Solution BS (conc.).
845-KS-1120050	Add 72 ml ethanol to 8 ml Washing Solution BS (conc.).
845-KS-1120250	Add 162 ml ethanol to 18 ml Washing Solution BS (conc.).

- Centrifugation steps should be carried out at room temperature.
- Preheat the Elution Buffer at 60 °C.

9 Protocols for DNA isolation from blood samples

9.1 Protocol 1: Isolation from 200 µl 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-purifies 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 to a Spin Filter located in a Receiver Tube using the pipette 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 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 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 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 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 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.

9.2 Protocol 2: Isolation from 400 µl samples

IMPORTANT NOTE
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 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-purifies 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 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 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 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 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 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 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.

10 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 at minimum -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

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