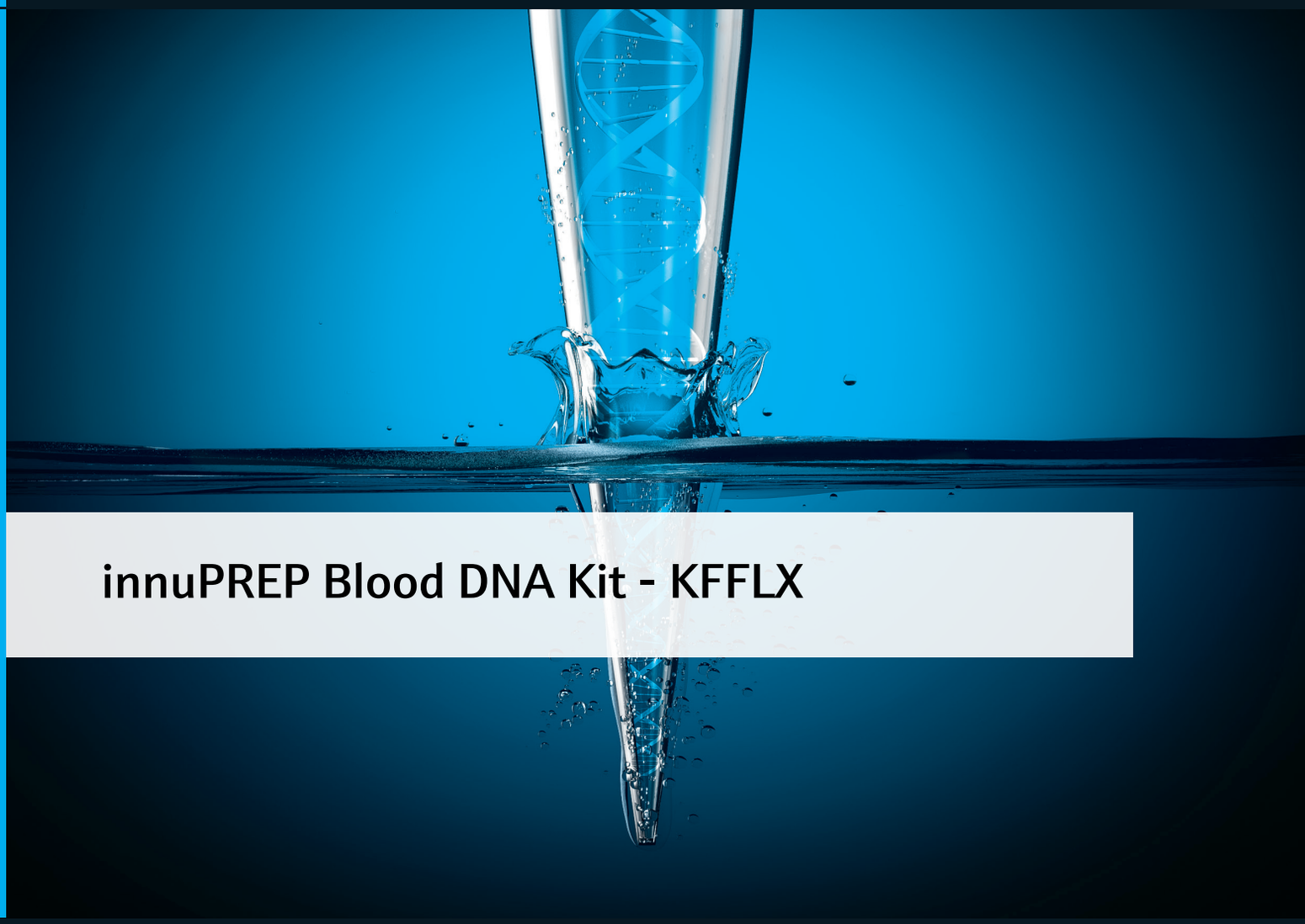
Instructions for Use
Life Science Kits & Assays



innuPREP Blood DNA Kit - KFFLX

Innuscreen GmbH

Order No.:
845-KF-8196096 96 reactions
845-KF-8196480 480 reactions

Publication No.: HB_KF-8196_e_220404

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 Introduction

1.1 Intended use

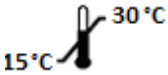
The **innuPREP Blood DNA Kit – KFFLX** has been designed for isolation of genomic DNA from blood samples. The extraction procedure is based on a new kind of chemistry (patent pending). The procedure combines lysis of starting material with subsequent binding of genomic DNA on surface modified magnetic particles. After washing steps the genomic DNA is eluted from the magnetic particles by using Elution Buffer. The extraction process is running in two steps (1. automated sample lysis followed by 2. automated nucleic acid extraction).

The extraction procedure takes place on the magnetic particle processor KingFisher FLEX and allows the parallel extraction of up to 96 samples. Extraction chemistry and extraction protocol are optimized to get maximum yield of high quality genomic DNA.

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 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** 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.

3 Safety precautions

NOTE
Read through this chapter carefully before use 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 to avoid any injuries. IST Innuscreen GmbH has not tested the liquid waste generated during use of the kit for potential residual infectious components. This case is highly unlikely but cannot be excluded completely. 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.

4 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival the **innuPREP Blood DNA Kit – KFFLX** should be stored dry at room temperature (15 °C to 30 °C).

Store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

Store **MAG Suspension** at 4 °C to 8 °C.

When stored as recommended, 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 solve these precipitates by careful warming.

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 kit was 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 Virus DNA/RNA Kit or other products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 30 9489 3380. For other countries please contact your local distributor.

6 Product use and warranty

The user is responsible to validate the performance of the IST Innuscreen GmbH kits for any particular use 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 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!

7 Kit components

7.1 Included kit components

	Σ 96	Σ 480
REF	845-KF-8196096	845-KF-8196480
MAG Suspension	5.5 ml	3 x 9 ml
Lysis Solution CLS	40 ml	180 ml
Washing Solution HS (conc.)	30 ml	2 x 70 ml
Washing Solution LS (conc.)	36 ml	180 ml
Washing Solution D	60 ml	250 ml
Proteinase K	for 2 x 1.5 ml working solution	for 7 x 1.5 ml working solution
Elution Buffer	25 ml	5 x 25 ml
KF96 Tip Comb with DW Plate	1	5 x 1
KF96 DW Plate	5	5 x 5
KF96 Elution Plate	1	1 x 5
Manual	1	1

7.2 Components not included in the kit

- 1.5 ml reaction tubes
- 96-99.8% ethanol (molecular biology grade, undenatured)
- ddH_2O for dissolving Proteinase K
- Isopropanol

8 Initial steps before starting

- Add the indicated amount of absolute ethanol to each bottle **Washing Solution HS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KF-8196096	Add 30 ml ethanol to 30 ml Washing Solution HS (conc.).
845-KS-8196480	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

- Add the indicated amount of absolute ethanol to each **bottle Washing Solution LS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KF-8196096	Add 144 ml ethanol to 36 ml Washing Solution HS (conc.).
845-KS-8196480	Add 720 ml ethanol to 180 ml Washing Solution HS (conc.).

- Add 1.5 ml of ddH_2O to each vial of Proteinase K, mix thoroughly and store as described above.
- Centrifugation steps should be carried out at room temperature
- Avoid freezing and thawing of starting material

9 Step I: Sample lysis for isolation of genomic DNA

NOTE
The extraction protocol is based on two automated runs:

Step I: Sample lysis on King Fisher FLEX

Step II: Extraction of gDNA on KingFisher FLEX

9.1 Isolation of genomic DNA from whole blood samples

1. Label one KF96 DW Plate (Deep Well Plate) with “Lysis Plate” and transfer **200 µl whole blood** into the wells.
2. Add **300 µl Lysis Solution CLS and 20 µl Proteinase K** to each well containing a sample.

   Follow the manual with chapter 9.2 “Settings of KingFisher FLEX and automated lysis run” on page 10.

9.2 Settings of KingFisher FLEX and automated lysis run

1. Label the KF96 Tip Comb with DW Plate with “Tip Comb”
2. Switch on KingFisher FLEX
3. Select the protocol “Blood Lysis”
4. Follow the instructions shown on the display and load the KF96 Tip Comb and KF96 DW Plate successively
   - Tip Comb
   - Lysis Plate
5. Start the automated sample lysis.

NOTE
After sample lysis protocol plate “Tip Comb” and “Lysis Plate” will further be used.

10 Step II: Extraction of genomic nucleic acids

10.1 Pre-filling of DW Plates and 96 Plate

NOTE
During sample lysis label the KF96 DW Plates and pre-fill all need-ed buffers into the wells of the KF96 DW Plates and the KF96 Elution Plate as described below!

KF96 DW Plate	Buffer
Binding Plate	After lysis protocol remove the "Lysis Plate" and plate "Tip Comb" from the KingFisher FLEX. Add 430 µl of Isopropanol and 50 µl MAG Suspension to each well containing the sample. **NOTE** It is important to mix the MAG Suspension by vigorous shaking or vortexing before use (approx. 30 sec)!
Washing Plate 1	500 µl Washing Solution HS
Washing Plate 2	800 µl Washing Solution LS
Washing Plate 3	800 µl Washing Solution LS
Washing Plate 4	500 µl Washing Solution D

KF96 Elution Plate	Buffer
Elution Plate	200 µl Elution Buffer

10.2 Settings of KingFisher FLEX and automated extraction run

1. Switch on KingFisher FLEX
2. Select protocol “Blood_Mini_P_KFFLX”
3. Follow the instructions shown on the display and load the KF96 Tip Comb, KF96 DW Plates and KF96 Elution Plate successively
   - KF96 Tip Comb (re-used from Step I)
   - KF96 Elution Plate
   - Washing Plate 4
   - Washing Plate 3
   - Washing Plate 2
   - Washing Plate 1
   - Lysis Plate (containing lysed sample, Isopropanol and MAG Suspension)
4. Start the automated extraction.

NOTE

1. After finishing the extraction protocol, the KF96 Elution Plate contains the extracted genomic DNA. Store the DNA under adequate conditions. We recommend storing the extracted DNA at -80 °C.
2. If the DNA contains carryover of magnetic particles, place the plate on a magnet or centrifuge the plate at maximum speed for 3 minutes and pipette the supernatant with DNA into a new 96 Plate.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted genomic DNA	
No extracted DNA	No magnetic beads added. Ensure **MAG Suspension** has mixed well before use.
Insufficient lysis of starting material.	Ensure to use the required volume of **Proteinase K**.
Elution volume too high.	Decrease the elution volume. The suggested elution volume is 200 µl. Please note that lowering the elution volume will not necessarily increase the yield proportional!
Inadequate extraction.	Inhibiting substances in starting material. Please use the kit only for samples that match requirements. Use internal controls for verification of extraction procedure.

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