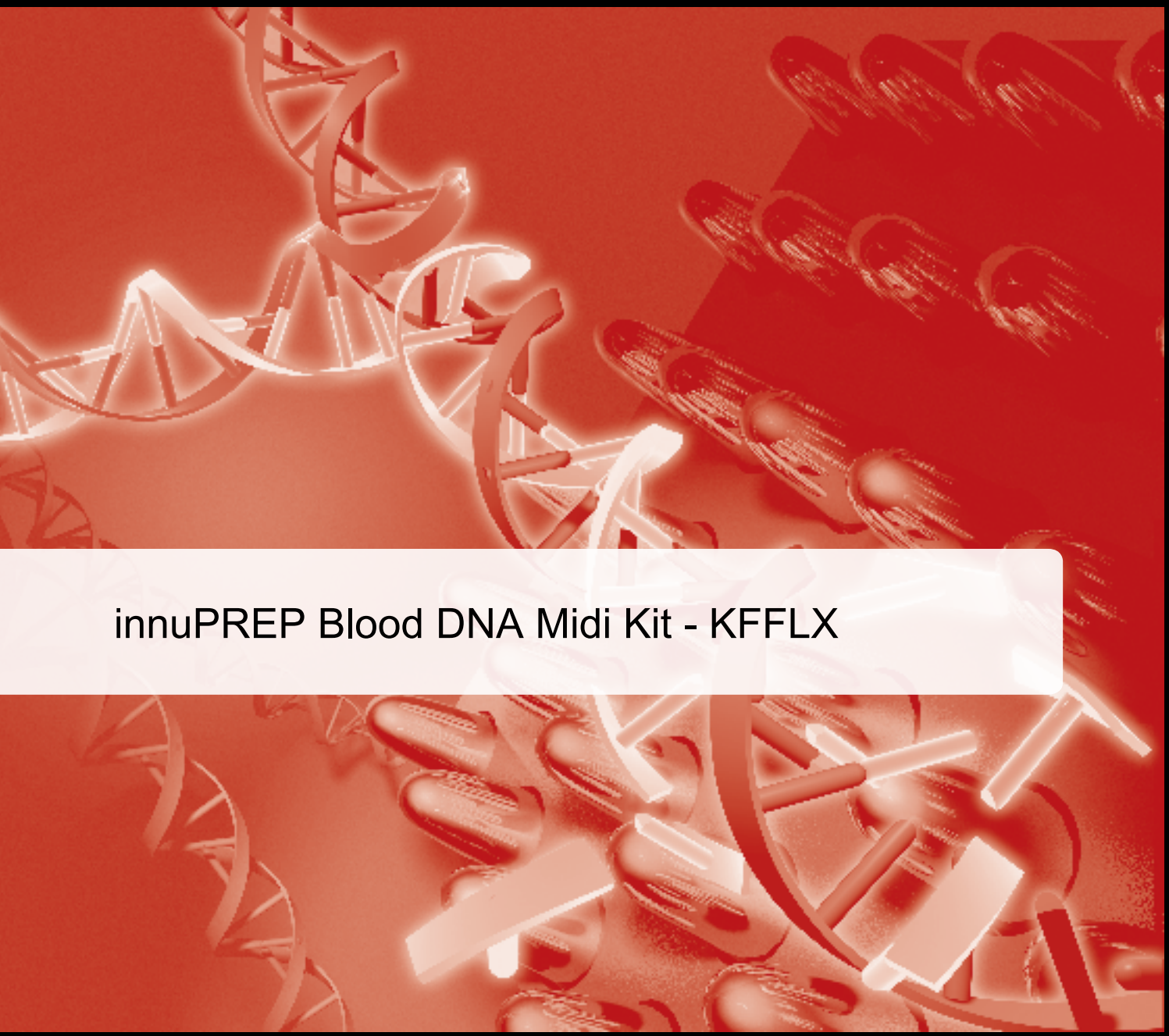
analytikjena

Manual



innuPREP Blood DNA Midi Kit - KFFLX

Life Science unlimited

Order No.:

845-KF-4396024	24 reactions
845-KF-4396120	120 reactions

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Contents

1 Introduction

1.1 Intended use

The has been designed for isolation of genomic DNA from blood samples up to 1 ml. 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 24 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 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:

REF
Catalogue number

N

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.

Used by
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 device 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. 4).
- 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 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. Analytik Jena AG has not tested the liquid waste generated during using 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. Observe 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, please ask for the material safety data sheet (MSDS):

3 Storage conditions

The innuPREP Blood DNA Midi Kit - KFFLX should be stored dry, at room temperature (14 °C – 25 °C) and is stable for at least 6 months under these conditions. 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. For further information see table kit components (→ "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. The components of each innuPREP Blood DNA Midi Kit - KFFLX were tested by isolation of DNA and subsequent PCR.

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 Midi Kit - KFFLX 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 user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, 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 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 the MAG Suspension S at 4 °C – 8 °C.

Store lyophilized Proteinase K at 4 °C!

Divide dissolved Proteinase K into aliquots and storage at – 20 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!

Storage conditions

All other components are stored at room temperature.

	Σ 24	Σ 120
REF	845-KF-4396024	845-KF-4396120
MAG Suspension S	3x 1 ml	2 x 5,5 ml
Lysis Solution BLB	30 ml	150 ml
Proteinase K	For 2 x 1.5 ml working solution	For 9 x 1.5 ml working solution
Washing Solution HS	30 ml (final vol. 60 ml)	2 x 70 ml (final vol. 2x 140 ml)
Washing Solution LS	36 ml (final vol. 180 ml)	180 ml (final vol. 900 ml)
Washing Solution AS	70 ml (final vol. 140 ml)	350 ml (final vol. 700 ml)
Washing Solution D (ready-to-use)	2 x 60 ml	2 x 250 ml
Elution Buffer	25 ml	3 x 25 ml
24 Tip Comb with 24 DW Plate	1	5
24 DW Plate (8.0 ml)	7	35
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	Σ 24	Σ 120
REF	845-KF-4396024	845-KF-4396120
Initial steps	Washing Solution HS: • Add 30 ml of 96 – 99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! Washing Solution LS: • Add 144 ml of 96 – 99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed! Washing Solution AS: • Add 70 ml of 96 – 99.8 % ethanol to the bottle Washing Solution AS, mix thoroughly and keep the bottle always firmly closed! Proteinase K: • Dissolve Proteinase K by addition of 1.5 ml ddH_2O, mix thoroughly and store as described above.	Washing Solution HS: • Add 70 ml of 96 – 99.8 % ethanol to each bottle of Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! Washing Solution LS: • Add 720 ml of 96 – 99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed! Washing Solution AS: • Add 350 ml of 96 – 99.8 % ethanol to the bottle Washing Solution AS, mix thoroughly and keep the bottle always firmly closed! Proteinase K: • Dissolve Proteinase K by addition of 1.5 ml ddH_2O, mix thoroughly and store as described above.

7 Recommended steps before starting

- Ensure that the Proteinase K, Washing Solution HS and Washing Solution LS have been prepared according to the instruction (→ "Kit components", p. 7).
- Centrifugation steps should be carried out at room temperature
- Avoid freezing and thawing of starting material
- Mix Lysis Solution BLB before using well, to get a homogenous solution!

8 Components not included in the kit

- 1.5 ml reaction tubes
- 96 – 99.8 % ethanol

  Note: Use only absolute/pure ethanol, NO methylated or denatured alcohol!
- Isopropanol

Important 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 Step I: Sample lysis for isolation of genomic DNA

Note

Mix Lysis Solution BLB before using well, to get a homogenous solution!

9.1 Protocol: Isolation of genomic DNA from whole blood samples

1. Label one 24 DW Plate (Deep Well Plate) with “Lysis Plate” and transfer **1.0 ml whole blood** into the wells.
2. Add **1.0 ml Lysis Solution BLB and 100 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 9.

9.2 Settings of KingFisher FLEX and automated lysis run

1. Label the 24 Tip Comb with 24 DW Plate with “Tip Comb”
2. Switch on KingFisher FLEX
3. Select the protocol “Blood_Lysis_Midi”
4. Follow the instructions shown on the display and load the Tip Comb and 24 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 24 Plate

> **Note**
> During sample lysis label the DW Plates and pre-fill all needed buffers into the wells of the DW Plates and the 24 Plate as described below!

DW Plate	Buffer
Binding Plate	After lysis protocol remove the “Lysis Plate” and plate “Tip Comb” from the KingFisher FLEX. Add 1.0 ml of Isopropanol and 90 µl MAG Suspension S to each well containing the sample. **Note!** It is important to mix the MAG Suspension S by vigorous shaking or vortexing before use (approx. 30 sec)!
Washing Plate 1	2.0 ml Washing Solution HS
Washing Plate 2	2.0 ml Washing Solution AS
Washing Plate 3	3.0 ml Washing Solution AS
Washing Plate 4	4.0 ml Washing Solution LS
Washing Plate 5	3.0 ml Washing Solution D

DW Plate	Buffer
Elution Plate	500 µl Elution Buffer

10.2 Settings of KingFisher FLEX and automated extraction run

1. Switch on KingFisher FLEX
2. Select protocol “Blood_DNA_Midi_KFFLX”
3. Follow the instructions shown on the display and load the Tip Comb, 24 DW Plates and 24 Plate successively
    - Tip Comb (re-used from Step I)
    - Elution Plate
    - Washing Plate 5
    - Washing Plate 4
    - Washing Plate 3
    - Washing Plate 2
    - Washing Plate 1
    - Bidning Plate (containing lysed sample, Isopropanol and MAG Suspension S)
4. Start the automated extraction.

Note

1. After finishing the extraction protocol, the 24 Plate (Elution Plate) contains the extracted genomic DNA. Store the DNA under adequate conditions. We recommend to store 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 24 Plate or 1.5 ml reaction tubes.

11 Related Products

Name	Amount	Order No.
Nucleic acid purification		
innuPREP Proteinase K	6 mg	845-CH-0010006
	30 mg	845-CH-0010030
Products for PCR & Gel Electrophoresis		
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
innuTaq RED DNA Polymerase (1 U/µl)	500 U	845-EZ-2000500
innuTaq Hot-A DNA Polymerase (5 U/µl)	500 U	845-EZ-3000500
innuTaq UltraPure DNA Polymerase (5 U/µl)	500 U	845-EZ-6000500
50x inNucleotide Mix (12,5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
25 mM $MgCl_2$ - Solution	3x 1.5 ml	845-AS-1000015
50 mM $MgCl_2$ - Solution	3x 1.5 ml	845-AS-1010015
PCR-grade H_2O	2.0 ml	845-AS-1800002
	5x 2.0 ml	845-AS-1800010
innuMIX rapidPCR MasterMix	100 rxn	845-AS-1600100
	200 rxn	845-AS-1600200
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 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3x 1.0 ml	845-ST-3010003
	6x 1.0 ml	845-ST-3010006
6x Loading Dye Orange G	3x 1.0 ml	845-ST-4010003
	6x 1.0 ml	845-ST-4010006
Products for qPCR		
innuMIX qPCR MasterMix Probe	100 rxn	845-AS-1200100
	200 rxn	845-AS-1200200
innuMIX qPCR MasterMix SyGreen	100 rxn	845-AS-1300100
	200 rxn	845-AS-1300200

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