Instructions for Use
Life Science Kits & Assays

innuPREP Stool DNA Kit - KF96 & KFFLX

analytikjena
An Endress+Hauser Company

Order No.:
845-KF-7015096 96 reactions
845-KF-7015480 480 reactions

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1 Introduction

1.1 Intended use

The innuPREP Stool DNA Kit - KF96 & KFFLX has been designed for isolation of bacterial DNA from stool samples. The extraction procedure is based on a new kind of chemistry. The procedure combines lysis of starting material with subsequent binding of bacterial DNA on surface modified magnetic particles. After washing steps the DNA is eluted from the magnetic particles. The extraction process is running in two steps (1. sample lysis followed by 2. automated nucleic acid extraction).

The extraction procedure takes place on the magnetic particle processor KingFisher 96 or 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 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 Stool DNA Kit - KF96 & KFFLX should be stored dry, at room temperature (15 °C–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 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 innuPREP Stool DNA Kit - KF96 & KFFLX were tested by isolation of bacterial DNA and subsequent PCR.

We reserve the right to change or modify our products to enhance there performance and design. If you have any questions or problems regarding any aspects of the innuPREP Stool DNA Kit - KF96 & 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!

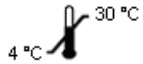
6 Kit components

Important

Store the MAG Suspension at 4 °C–8 °C.

Store lyophilized Proteinase K at 4 °C–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 96	Σ 480
REF	845-KF-7015096	845-KF-7015480
MAG Suspension	3 x 1.0 ml	2 x 5.5 ml
Lysis Solution SLS	125 ml	3 x 220 ml
Binding Solution SBS	50 ml	240 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. 2 x 140 ml)
Washing Solution MS	60 ml (final vol. 200 ml)	240 ml (final vol. 800 ml)
Elution Buffer	25 ml	3 x 25 ml
Pre-Filter	1 x 96	5 x 96
Receiver Tube (2.0 ml)	2 x 50	10 x 50
96 Tip Comb with 96 DW Plate	1	5
96 DW Plate (2.0 ml)	4	20
Elution Plate 96 Plate (200 µl)	1	5
Manual	1	1

	Σ 96	Σ 480
REF	845-KF-7115096	845-KF-7115480
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 MS: • Add 140 ml of 96 – 99.8 % ethanol to the bottle Washing Solution LS, 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 MS: • Add 560 ml of 96 – 99.8 % ethanol to the bottle Washing Solution LS, 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

- Heat thermal mixer or water bath at needed temperature (95°C and 70°C)
- Ensure that the Proteinase K, Washing Solution HS and Washing Solution MS 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

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!
- ddH_2O

9 Lysis protocol for Isolation of bacterial DNA from stool samples

Note

The lysis of the starting material is a preliminary manual processing step.

Maximum amount of stool samples (fresh or frozen) is 200–400 mg. If the sample is liquid, pipet 200–400 µl into 2.0 ml Safe-Lock tube. Cut the end of the pipet tip to make pipetting easier.

1. Weight **200–400 mg of stool sample (fresh or frozen)** into a 2.0 ml Safe-Lock tube.
2. Add **1.2 ml Lysis Solution SLS** to each stool sample, mix vigorously by pulsed vortexing for 1 minute to get a homogeneous suspension.
3. Incubate the sample for 15 minutes at 95 °C in a thermomixer under continuously shaking at 900 rpm.

   Important note: The incubation step at 95 °C will maximize the amount of bacterial DNA, because of a very efficient destruction of the cell walls of e.g. gram+ bacteria.
4. After lysis place a Pre-Filter into a Receiver Tube (2.0 ml) and transfer 800 µl of the sample volume to the Pre-Filter.
5. Centrifuge the Pre-Filter with Receiver Tube (2.0 ml) at 10.000 x g (12.000 rpm) for 2 minutes. Remove and discard the Pre-Filter, transfer the filtrate into a 1.5 ml reaction tube.
6. Add **25 µl Proteinase K** to the sample, mix shortly.
7. Incubate the sample for 10 minutes at 70 ° in a thermomixer under continuously shaking at 900 rpm.
8. After lysis time the lysed sample (450 µl) will be transferred carefully into the well of the Deep Well Plate 2 (as described below).

   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 every 10 minutes during the incubation. No shaking will reduce the lysis efficiency.

   Important note: The lysis step should be finished if the material is completely lysed.
9. Follow the manual with chapter 10 “Preliminary steps of KingFisher 96 or KingFisher FLEX” on page 10.

10 Preliminary steps of KingFisher 96 or KingFisher FLEX

10.1 Pre-filling of DW Plates and 96 Plate

Note
During sample lysis label the DW Plates and pre-fill all needed buffers into the wells of the DW Plates and the 96 Plate as described below!

DW Plate	Buffer
Deep Well Plate 1	Contains the KingFisher 96 tip comb for DW magnets
Deep Well Plate 2	Add 20 µl MAG Suspension and 450 µl Binding Solution SBS to each well. **Note!** It is important to mix the MAG Suspension by vigorous shaking or vortexing before use (approx. 30 sec)!
Deep Well Plate 3	500 µl Washing Solution HS
Deep Well Plate 4	500 µl Washing Solution MS
Deep Well Plate 5	800 µl Washing Solution MS

96 Plate	Buffer
Elution Plate	150 µl Elution Buffer

10.2 Settings of KingFisher 96 or KingFisher FLEX and automated extraction run

1. Switch on KingFisher 96 or KingFisher FLEX
2. Select protocol “INNU_Stool_KF96” for KingFisher 96 or “INNU_Stool_KFFLX” for KingFisher FLEX
3. Press [Start]
4. Follow the instructions shown on the display and load the Tip Comb, 96 DW Plates and 96 Plate successively
    - Deep Well Plate 1 with Tip Comb
    - Elution Plate
    - Deep Well Plate 5
    - Deep Well Plate 4
    - Deep Well Plate 3
    - Deep Well Plate 2 (containing lysed sample, Binding Solution SBS and MAG Suspension)
4. Start the automated extraction.

Note

1. After finishing the extraction protocol, the 96 Plate (Elution Plate) contains the extracted DNA. Store the DNA under adequate conditions. We recommend to store the extracted DNA at 4 °C–8 °C or for long term storage at -22 °C to -18 °C.

2. If the extracted 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 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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Subject to changes in design and scope of delivery as well as further technical development.