analytikjena

Manual

innuPREP Tissue DNA Kit - KF96 & KFFLX

Life Science unlimited

Order No.:

845-KF-7115096	96 reactions
845-KF-7115480	480 reactions

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Contents

1 Introduction

1.1 Intended use

The innuPREP Tissue DNA Kit - KF96 & KFFLX has been designed for isolation of genomic DNA from different kinds of starting material. The extraction procedure is based on a new kind of chemistry. The procedure combines lysis of starting material with subsequent binding of 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 Tissue DNA Kit - KF96 & 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 innuPREP Tissue DNA Kit - KF96 & KFFLX were tested by isolation of 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 Tissue 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!

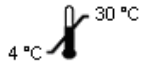
6 Kit components

Important

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

Store lyophilized Proteinase K at 4 °C!

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 96	Σ 480
REF	845-KF-7115096	845-KF-7115480
MAG Suspension	5,5 ml	3 x 9 ml
Lysis Solution QPT	50 ml	240 ml
Binding Solution SBS	50 ml	240 ml
Proteinase K	For 2 x 1.5 ml working solution	For 7 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)
Elution Buffer	25 ml	5 x 25 ml
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 LS: • Add 144 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 LS: • Add 720 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 (50°C; optional 90°C)
- 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

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
- Optional: Isopropanol (molecular biology grade)

9 Lysis protocols for different starting materials

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

9.1 Protocol 1: DNA isolation from tissue samples or rodent tails

> **Note**
> Max. amount of tissue samples is 30 mg. In case of high viscous sample (e.g. spleen) use max. 10 mg.
> Max. rodent tails: 0.4 – 0.8 cm

1. Cut **max. 30 mg of tissue sample or rodent tail** into small pieces and place the tissue in a 1.5 ml or 2.0 ml reaction tube.
2. Add **400 µl Lysis Solution QPT, 20 µl Proteinase K and 3 µl RNase A** (stock solution 100 mg/ml; not included in the kit), mix vigorously by pulsed vortexing for 5 sec.
3. Incubate at 50 °C (approx. 0.5 – 2 h for tissue sample and approx. 3 h for rodent tails).

   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.

4. Label one 96 DW Plate (Deep Well Plate) with “Deep Well Plate 2” and transfer **400 µl Binding Solution SBS** into the wells. Add **400 µl** of the **sample** to each well containing **Binding Solution SBS**.
5. Follow the manual with chapter 10 “Preliminary steps of KingFisher 96 or KingFisher FLEX” on page 11.

9.2 Protocol 2: Isolation of DNA from paraffin embedded tissue sample

1. Place the **FFPE material** (approx. 2x 5 µm; optional more starting material) into a 1.5 ml or 2.0 ml reaction tube.
2. Add **400 µl Lysis Solution QPT and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C for 1 hour.

   Important note: The FFPE material have to be completely covered by Lysis Solution QPT! If not, reduce the starting material.

6. After lysis step place the sample into a thermal mixer pre-heat to 90 °C and incubate the sample for 1 hour.

   Important note: Do not place the sample into the thermal mixer, before the temperature of 90 °C is achieved!

7. Label one 96 DW Plate (Deep Well Plate) with “Deep Well Plate 2” and transfer **400 µl Isopropanol** into the wells. Add **400 µl** of the **sample** to each well containing **Isopropanol**.
8. Follow the manual with chapter 10 “Preliminary steps of KingFisher 96 or KingFisher FLEX” on page 11.

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 50 µl MAG Suspension to each well containing the sample and Binding Solution SBS or Isopropanol respectively. **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	800 µl Washing Solution LS
Deep Well Plate 5	800 µl Washing Solution LS

96 Plate	Buffer
Elution Plate	200 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_Tissue_KF96” for KingFisher 96 or “INNU_Tissue_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 or Isopropanol respectively 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 or for longterm storage at –20 °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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