Instructions for Use
Life Science Kits & Assays

innuPREP Stool DNA Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-7010010 10 reactions
845-KS-7010050 50 reactions

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Contents

1 Introduction

1.1 Intended use

The kit has been designed as a tool for isolation of bacterial and host DNA from stool samples. 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.

The kit is for research use only!

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

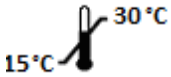
For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

REF

Catalogue number

Content

Contains sufficient reagents for <N> tests



Storage conditions

Store at room temperature

Consult instructions for use

This information must be observed to avoid improper use of the kit and the kit components.

Use by

Lot number

The number of the kit charge.

Manufactured by

For single use only

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. 3).
- 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 immediately with a large amount of water.

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.

Clinical sample must always be considered as potentially infectious. Samples from risk patients must always be labeled and handled under consequent safety conditions. 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 isolation should be free of DNases.

Below the European Community risk and safety phrases for the components of the innuPREP Stool DNA Kit to which they apply, are listed.

Binding Solution SBS:	contains 2-propanol; highly flammable, irritant (R11, 36, 67, 7, 16, S24/25/26)
Proteinase K:	irritant, sensitizing. Risk and safety phrases: R36/37/38-42, S22-24-26-37/38
Washing Solution HS:	contains guanidine thiocyanate: harmful. Risk and safety phrases: R20/21/22-32, S13-26-36-46

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 Safety Data Sheets (SDS).

3 Storage conditions

Store lyophilized Proteinase K at 4 °C to 8 °C! Divide dissolved Proteinase K into aliquots and store at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!

All other components of the innuPREP Stool DNA 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 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual.

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 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 kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the intended use (→ "Intended use" p. 3) and described in the summary (→ "Extraction procedure" p. 10).

All plastic components and the chemistry are disposable products. When changing the starting material or the flow trace, no guarantee of the operability is issued. Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols then those described below. 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 equivalent regulations required in other countries.

All products sold by the 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.

6 Kit components

	Σ 10	Σ 50
REF	845-KS-7010010	845-KS-7010050
Lysis Solution SLS	12 ml	60 ml
Binding Solution SBS	5 ml	20 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution
Washing Solution HS (conc.)	5 ml (final volume 10 ml)	20 ml (final volume 40 ml)
Washing Solution MS (conc.)	3 ml (final volume 10 ml)	15 ml (final volume 50 ml)
Elution Buffer	2 x 2 ml	15 ml
Pre-filter	10	50
Spin Filter	10	50
Receiver Tubes (2.0 ml)	60	6 x 50
Elution Tubes (1.5 ml)	10	50
Manual	1	1
Initial steps	• Add 5 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 7 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 0.3 ml of ddH_2O, mix thoroughly and store as described below!	• Add 20 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 35 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described below!

Important

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 components besides Proteinase K are stored at room temperature.

7 Recommended steps before starting

- Heat thermal mixer or water bath at the needed temperature (95 °C and 70 °C)
- Ensure that the Washing Solution HS, Washing Solution MS and Proteinase K have been prepared according to the instruction (→ "Kit components" p. 8).
- 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 and 2.0 ml reaction tubes
- 96 – 99.8 % ethanol
- ddH_2O

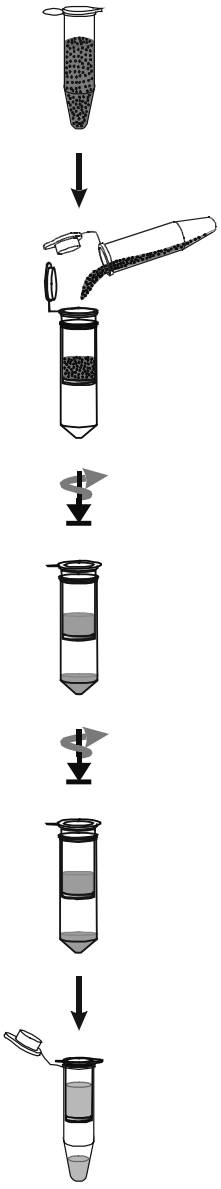
9 Extraction procedure

9.1 Summary

The kit has been designed as a tool for isolation of bacterial and host DNA from stool samples. The kit combines a very efficient lysis of starting materials following a pre-filtration step to remove unlysed particles. After pre-filtration the sample will be transferred onto a spin filter unit and the DNA will be bound on the spin filter surface. After washing of the bound DNA, the DNA will be eluted from the spin filter and is ready to use for further downstream applications.

The recovery of DNA and the quality are excellent. Extracted DNA is available approx. 15 minutes after lysis of the starting material. The isolated DNA is suitable for all downstream applications commonly used.

9.2 General extraction principle



Lysis of starting material and pre-filtration using a Pre-filter (mauve)

Binding of DNA onto Spin Filter

Washing of the bound DNA

Elution of DNA

10 Product specifications

1. **Starting material:**

   Stool samples from different origins (200–400 mg)

2. **Time for isolation:**

   Approximately 30–45 minutes depending on the kind of application

3. **Typical yield:**

   Not determined. Sufficient DNA for enzymatic amplification reactions.

The extracted gDNA can be used for a wide range of different molecular biology applications.

11 Protocol 1: Isolation of bacterial DNA from stool samples

1. Sample homogenization and pre-lysis

Weigh **200-400 mg** of **stool sample** (fresh or frozen) into a 2.0 ml safe-lock tube.

Note: If the sample is liquid pipette 200-400 µl into the 2.0 ml safe-lock tube. Cut the end of the pipette tip to make pipetting easier.

Add **1.0 ml Lysis Solution SLS** to each stool sample. Vortex vigorously for 1 minute to get a homogeneous suspension.

Incubate the sample for 15 minutes at 95 °C in a thermal mixer under continuous shaking at 900 rpm.

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.

2. Sample cleanup

Transfer the sample onto a Pre-filter located in a 2.0 ml Receiver Tube. Transfer **650 µl** of the **sample volume** into the Pre-filter (mauve) unit. Centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes. Remove and discard the Pre-filter unit. Transfer the filtrate into a 1.5 ml reaction tube (not included in the kit).

3. Proteinase K digestion

Add **25 µl Proteinase K** to the sample and mix it shortly. Incubate the sample for 20 minutes at 70 °C in a thermal mixer under continuous shaking at 900 rpm.

4. Binding

Add **300 µl Binding Solution SBS** to the lysed sample (filtrate from step 3) and mix by pipetting up and down several times.

Note: It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

Apply **650 µ**l of the **sample** to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes.

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube and apply the residual sample to the Spin Filter.

Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes.

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

5. Washing step 1

Open the Spin Filter and add **600 µl Washing Solution HS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

6. Washing step 2

Open the Spin Filter and add **750 µl Washing Solution MS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

7. Removing of ethanol

Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

8. Elution

Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **100-200 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. A second elution step will increase the yield of extracted DNA.

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. For long time storage placing at –20 °C is recommended.

12 Protocol 2: Isolation of host DNA from stool samples

1. Sample homogenization and prelysis

Weigh **200-400 mg** of **stool sample** (fresh or frozen) into a 2.0 ml safe-lock tube.

Note: If the sample is liquid, pipette 200-400 µl into the 2.0 ml safe-lock tube. Cut the end of the pipette tip to make pipetting easier.

Add **1.0 ml Lysis Solution SLS** to each stool sample. Vortex vigorously for 1 minute to get a homogeneous suspension.

2. Sample cleanup

Transfer the sample onto a Pre-filter (mauve) located in a 2.0 ml Receiver Tube. Transfer **650 µl** of the **sample volume** into the Pre-filter unit. Centrifuge at 10.000 x g (~12.000 rpm) for 2 min. Remove and discard the Pre-filter unit. Transfer the filtrate into a 1.5 ml reaction tube (not included in the kit).

3. Proteinase K digestion

Add **25 µl Proteinase K** to the sample and mix it shortly. Incubate the sample for 20 minutes at 70 °C in a thermal mixer under continuous shaking at 900 rpm.

4. Binding

Add **300 µl Binding Solution SBS** to the lysed sample (filtrate from step 3) and mix by pipetting up and down several times.

Note: It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

Apply **700 µl** of the **sample** to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes.

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube and apply the residual sample to the Spin Filter.

Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes.

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

5. Washing step 1

Open the Spin Filter and add **600 µl Washing Solution HS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

6. Washing step 2

Open the Spin Filter and add **750 µl Washing Solution MS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

7. Removing of ethanol

Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

8. Elution

Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **100-200 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 6.000 x g (~8.000 rpm) for 1 minute. A second elution step will increase the yield of extracted DNA.

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. For long time storage placing at –20 °C is recommended.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
• Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
Low amount of extracted DNA	
• 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 SBS	Mix sample with Binding Solution SBS by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.

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