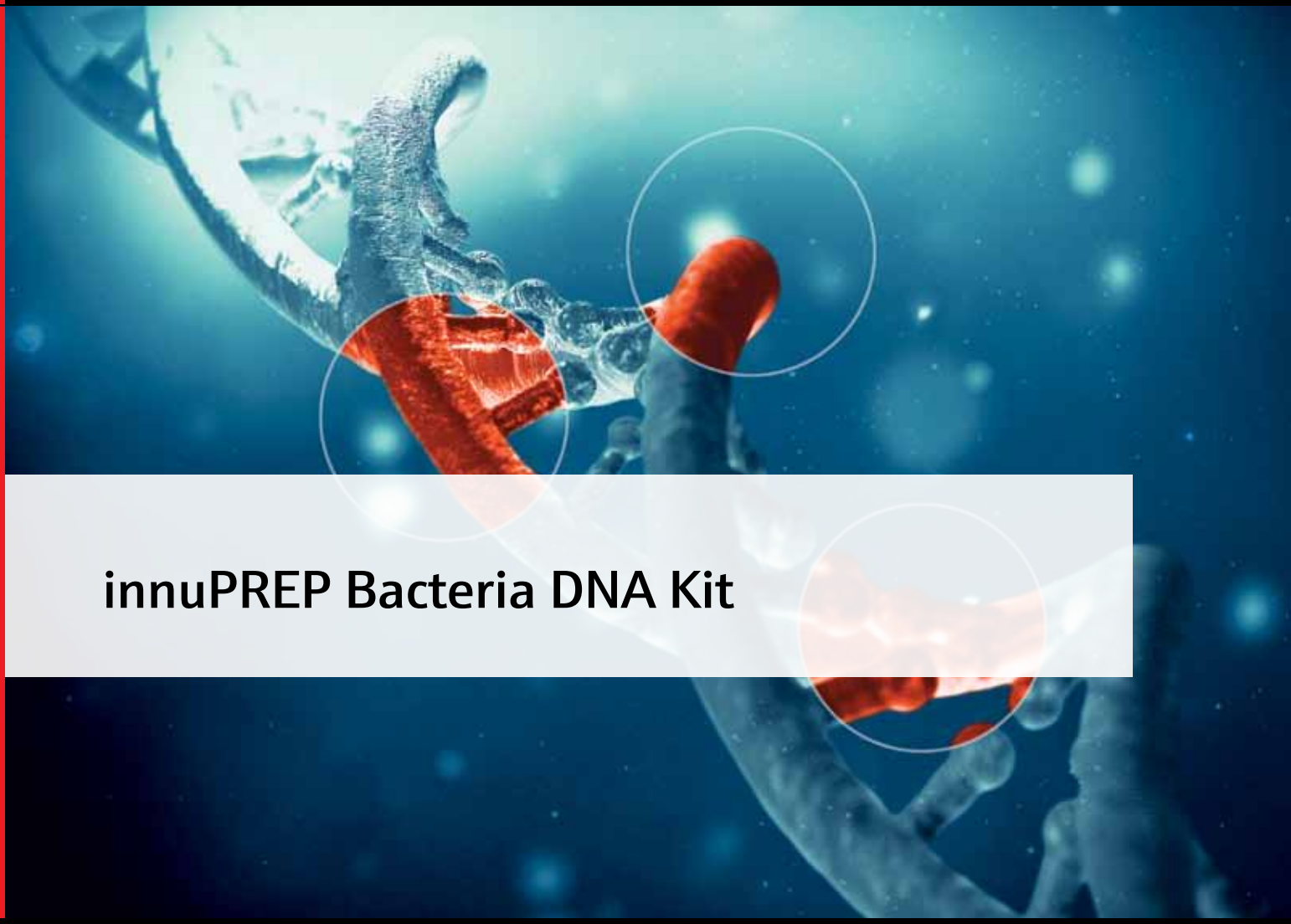
Instructions for Use
Life Science Kits & Assays



innuPREP Bacteria DNA Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-6010010 10 reactions
845-KS-6010050 50 reactions
845-KS-6010250 250 reactions

Publication No.: HB_KS-6010_e_180329

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Contents

1 Introduction

1.1 Intended use

The kit has been designed as a tool for isolation of DNA from culture pellets of gram+ and gram- bacteria. The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream application like amplification.

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:

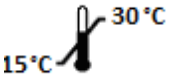
REF

Catalogue number

N

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 Bacteria DNA Kit to which they apply, are listed.

Binding Solution TBS:	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 Bacteria 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. The components of each innuPREP Bacteria DNA Kit were tested by isolation of bacterial DNA from bacterial cell pellet and subsequent target-amplification.

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 Bacteria 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.

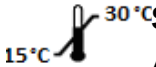
6 Kit components

	Σ 10	Σ 50	Σ 250
REF	845-KS-6010010	845-KS-6010050	845-KS-6010250
Lysis Solution TLS	2 x 2 ml	12 ml	60 ml
Binding Solution TBS	5 ml	25 ml	120 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution	for 5 x 1.5 ml working solution
Washing Solution HS (conc.)	3 ml	15 ml	70 ml
Washing Solution MS (conc.)	3 ml	15 ml	60 ml
Elution Buffer	2 ml	12 ml	60 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	40	4 x 50	20 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1
Initial steps	• Add 3 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 15 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!	• Add 70 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 140 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 to 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 components besides Proteinase K are stored at room temperature.

7 Recommended steps before starting

- Heat thermomixer or water bath at 37 °C and at 50 °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

- TE-Buffer
- Lysozym (stock solution 10 mg/ml)
- 1.5 ml tubes
- 2.0 ml tubes; optional
- 96–99.8 % ethanol
- ddH_2O

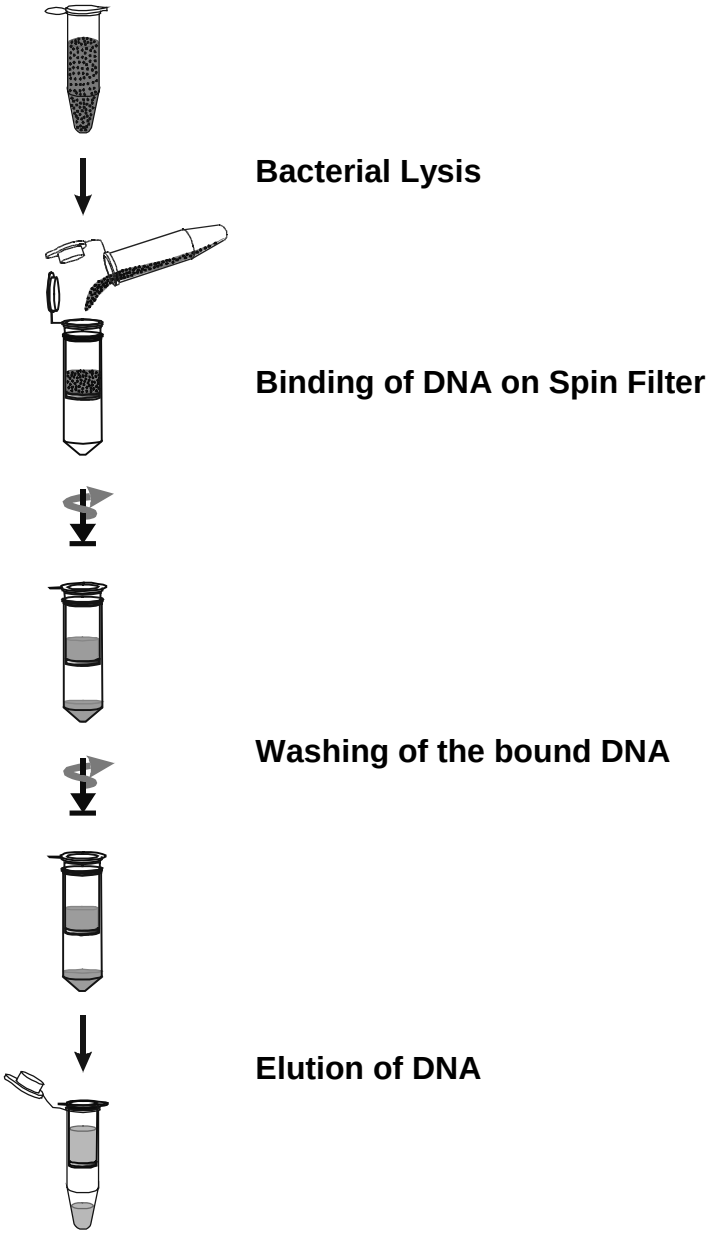
9 Extraction procedure

9.1 Summary

The kit has been designed as a tool for isolation of DNA from culture pellets of gram+ and gram- bacteria. The extraction procedure combines a lysis step with lysozyme following Proteinase K digestion and subsequent efficient binding of genomic DNA on a spin filter surface. The bound DNA will be washed and finally eluted.

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

9.2 General extraction principle



10 Product specifications

1. **Starting material:**
   - Culture pellets of gram+ and gram- bacteria
   - Up to 1 x 10^9 cells

2. **Time for isolation:**

   Approximately 45 min or approximately 15 minutes after the lysis step

3. **Binding capacity:**

   > 50 µg DNA

4. **Typical yield:**
   - The yield depends on the type of bacteria and on the amount of the starting material.
   - The typical yield is sufficient for amplification procedures.
   - Up to 35 µg

11 Protocol: DNA isolation from bacterial cell pellets after cultivation

1. Add **200 µl TE buffer** to the bacterial pellet and re-suspend the pellet completely. Add **15 µl Lysozym** (stock solution 10 mg/ml in TE buffer). Mix by pulsed vortexing for 5 sec. Incubate at 37 °C until the sample is lysed.

   Note: The lysis time depends very strong on type of starting material (e.g. gram+ or gram- bacteria). We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation.

2. Add **200 µl Lysis Solution TLS and 25 µl Proteinase K** to the sample, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C for 15 minutes.

   Note: We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally during incubation. No shaking will reduce the lysis efficiency!

3. If necessary, centrifuge the 1.5 ml tube at 10.000 x g (12.000 rpm) for 1 minute to spin down unlysed material. Transfer the supernatant into another 1.5 ml tube.

4. Add **400 µl Binding Solution TBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

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

5. Apply the sample to the Spin Filter 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.

6. Open the Spin Filter and add **500 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.

7. 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.

8. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
9. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50-100 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 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 TBS	Mix sample with Binding Solution TBS by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
• Too much Elution Buffer	Elute the DNA with lower volume of Elution Buffer.
RNA contaminations of extracted DNA	RNase A digestion

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