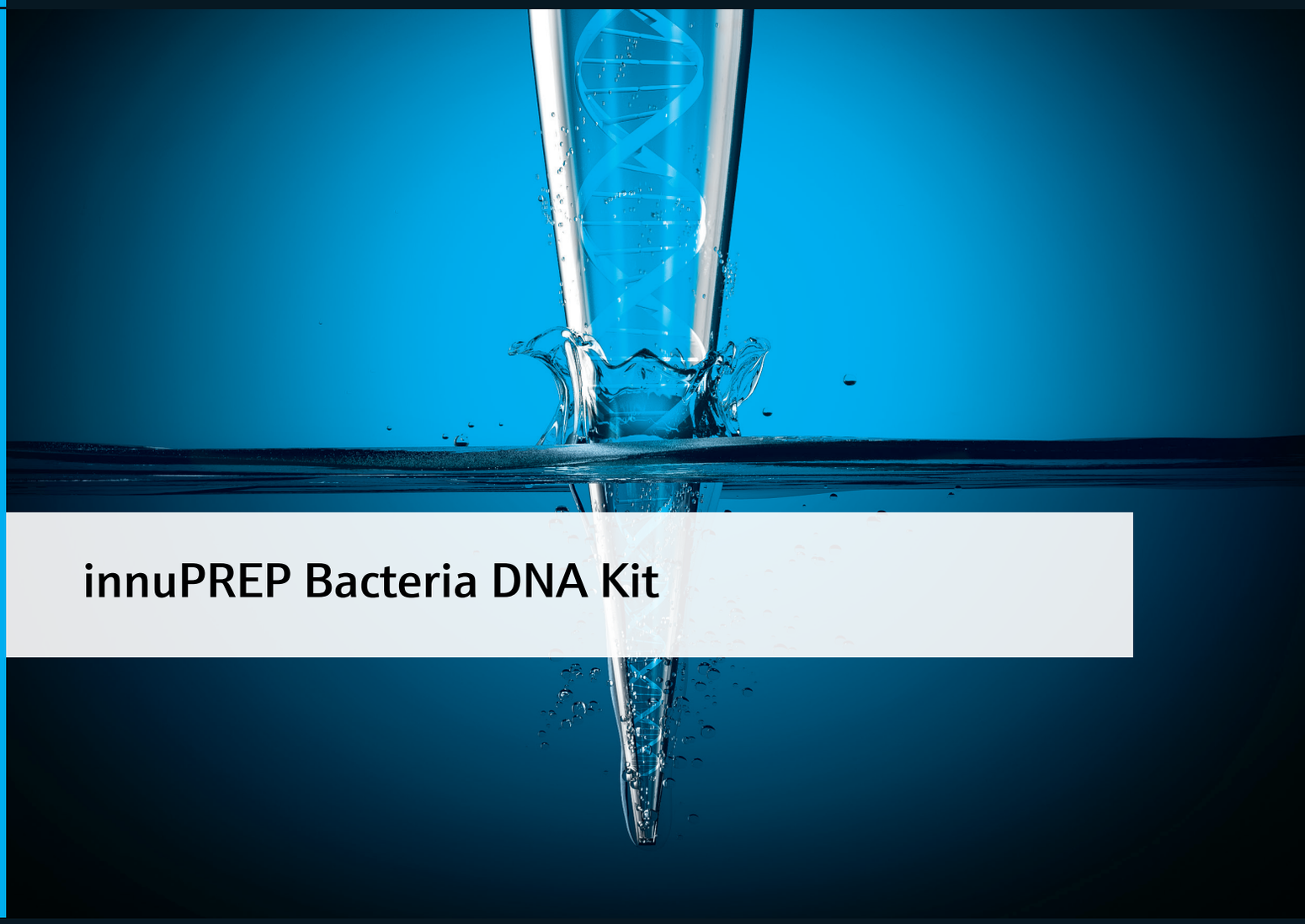
Instructions for Use
Life Science Kits & Assays



innuPREP Bacteria DNA Kit

Innuscreen GmbH

Order No.:

845-KS-6010010 10 reactions
845-KS-6010050 50 reactions
845-KS-6010250 250 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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1 Introduction

1.1 Intended use

The **innuPREP Bacteria DNA Kit** has been designed for isolation of DNA from Gram-positive and Gram-negative bacteria cells derived from liquid cultures.
The extraction procedure starts with centrifugation of bacterial cultures and a subsequent pre-lysis step. Especially when processing Gram-positive bacteria (e.g. *Staphylococcus* strains) this step significantly enhances the yield of bacterial DNA. The pre-lysis step is followed by Proteinase K digestion and subsequent DNA isolation using spin column technology.
By this means the innuPREP Bacteria DNA Kit allows a fast and efficient isolation of DNA from a broad spectrum of bacteria. The bacterial DNA is available in approximately 15 minutes after lysis of starting material. The isolated DNA is suitable for all downstream applications commonly used.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully before 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 and the kit

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

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15°C 30°C	**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.
	**Expiry date**
LOT	**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 kit 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 and the kit" p. 3).
- Work steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before 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 personnel 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. This kit could be used with potential infectious samples. 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. Follow 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 on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

Store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

All other components of the innuPREP Bacteria DNA Kit 2.0 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 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This kit has been produced and tested in an ISO 13485 certified facility.

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 Bacteria DNA Kit or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. 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 manual, (→ “Product specifications“ p. 8). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH 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 IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

6 Kit components

6.1 Included 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 4 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

6.2 Components not included in the kit

- 2.0 ml reaction tubes
- ddH2O for dissolving Proteinase K
- 96–99.8 % ethanol, non-denatured or methylated
- Lysozyme (10 mg/ml, 400 U/µl), Mutanolysin (0.4 U/µl) and Lysostaphin (0.4 U/µl) or alternatively innuPREP Bacteria Lysis Booster (IST Innuscreen, 845-KA-1000050)
- RNase A (10 mg/ml); optional
- TE Buffer

7 Product specifications

1. **Starting material:**

- Cultured pellets of Gram-positive and Gram-negative bacteria
- Up to 1 x 10^9 cells

2. **Time for isolation:**

- Approximately 15 minutes after lysis step

3. **Typical yield:**

- Depends on the type of bacteria and amount of starting material
- Binding capacity of Spin Filter columns > 50 µg DNA

8 Initial steps before starting

- Add the indicated amount of ddH_2O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-KS-6000010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-6000050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-KS-6000250	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of absolute ethanol to each bottle **Washing Solution HS (conc.)**, mix thoroughly. Store as described above. Always keep the bottle firmly closed.

845-KS-6000010	Add 3 ml ethanol to 3 ml Washing Solution HS (conc.).
845-KS-6000050	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
845-KS-6000250	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

- Add the indicated amount of absolute ethanol to each bottle **Washing Solution MS (conc.)**, mix thoroughly. Store as described above. Always keep the bottle firmly closed.

845-KS-6000010	Add 7 ml ethanol to 3 ml Washing Solution MS (conc.).
845-KS-6000050	Add 35 ml ethanol to 15 ml Washing Solution MS (conc.).
845-KS-6000250	Add 140 ml ethanol to 60 ml Washing Solution MS (conc.).

- Heat thermal mixer or water bath to 37 °C (later to 50 °C).
- Centrifugation steps should be carried out at room temperature.

9 Pre-lysis of bacterial cell walls

9.1 Collection of bacterial cells

1. Transfer the bacterial culture (volume depends on the concentration of starting material) into a tube 2.0 ml or 1.5 ml tube depends on initial volume.
2. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g). Discard the supernatant. Do not discard the pellet!
3. Resuspend the bacterial cell pellet in **170 µl TE-Buffer**.

9.2 Gram-negative bacteria

NOTE
Although Gram-negative bacteria do not require a pre-lysis step, using Lysozyme (not included in the kit) can enhance the efficiency of lysis.

4. Add 20 µl Lysozyme (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
5. Proceed with “Proteolytic lysis step” on p. 12.

9.3 Gram-positive bacteria

NOTE
Gram-positive bacteria require a pre-lysis step using Mutanolysin and/or Lysozyme (not included in the kit).

4. Add **20 µl Lysozyme** (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
5. Add **5 µl Mutanolysin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.

NOTE
Lysozyme and Mutanolysin exert synergistic activity. Using both enzymes

together will increase the yield of isolated nucleic acids.

6. Proceed with “Proteolytic lysis step” on p. 12.

9.4 Staphylococcus strains

NOTE
For pre-lysis of *Staphylococcus* the enzyme Lysostaphin is recommended (not included in the kit).

5. Add 1**0 µl Lysostaphin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
6. Proceed with “Proteolytic lysis step” on p. 12.

9.5 Alternative pre-lysis using innuPREP Bacteria Lysis Booster

NOTE
The innuPREP Bacteria Lysis Booster Kit has been developed for a highly efficient pre-lysis of bacterial cell walls by generating spheroblasts. This new mixture of different enzymes boost the lysis of all bacteria in particular the hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium*.

4. Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.
5. Add **20 µl enzyme mix** to the sample and vortex shortly. Incubate the sample for 30 minutes at 37 °C under continuous shaking.
6. Proceed with “Proteolytic lysis step” on p. 12.

10 Proteolytic lysis step

1. Add **200 µl Lysis Solution TLS** and **25 µl Proteinase K** to the sample and vortex the sample for 5 sec.
2. Incubate the sample for 15 minutes at 50 °C using a thermoshaker.
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. Continue with “Bacterial DNA extraction” on p. 12.

IMPORTANT

To remove RNA from sample (optional) add 1-2 µl RNase A (10 mg/ml), vortex shortly and incubate for 10 minutes at room temperature.

11 Bacterial DNA extraction

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

IMPORTANT

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

2. Apply the sample to the Spin Filter located in a 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.

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

4. 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 filtrate and reuse the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
5. 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 filtrate and reuse the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
6. Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
7. Place the Spin Filter into a 1.5 ml tube. Carefully open the cap of the Spin Filter and add **50–100 µl Elution Buffer**. Incubate at room temperature for 2 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 bacterial DNA). Elution with lower volumes of Elution Buffer increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -18 °C to -22 °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 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 was used for the elution step	Elute the DNA with lower volume
Degraded or sheared DNA	
Incorrect storage of starting material	Ensure that the starting material is frozen immediately after taking in liquid nitrogen or at -18 °C to -22° C! For long time storage continuously store at -78 °C to -82° C! Avoid thawing of the material.
Old starting material	Old material often contains degraded DNA. Repeat with fresh material.
RNA contamination	
Extracted DNA is contaminated with RNA	Perform an RNase A digestion

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