Instructions for Use
Life Science Kits & Assays

innuPREP Beer Bacteria DNA Kit

Innuscreen GmbH

Order No.:

845-KS-9000010	10 reactions
845-KS-9000050	50 reactions

Publication No.: HB_KS-9000_e_241106

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1 Introduction

1.1 Intended use

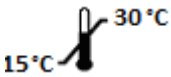
The **innuPREP Beer Bacteria DNA Kit** is designed for efficiently isolating bacterial DNA from beer samples, turbid liquids and bacteria shaking cultures.
The kit utilizes a patented DNA extraction technology that integrates an initial, highly efficient homogenization step with subsequent DNA binding to a spin filter. The process is facilitated by specially formulated buffers, which enable the isolation of high-quality DNA that is free from PCR inhibitors. This approach ensures the recovery of pure DNA, even from challenging samples such as turbid or dark beers.

For processing larger sample volumes (> 50 ml) the volume should be reduced to 20 ml using the **innuPREP TCT Target Concentration Kit Beer** (845-TC-0030010/50).

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** Catalogue number.
	**Content** Contains sufficient reagents for <N> tests.
	**Storage conditions** Store at room temperature, unless otherwise specified.
	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	**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 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).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before use 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 is designed to be handled only 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 is to be used with potential infectious human 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 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 Beer 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 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product 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 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 equivalent regulations required in other countries.

All products sold by the 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 Components included in the kit

	Σ 10	Σ 50
REF	845-KS-9000010	845-KS-9000050
Proteinase K	for 2 x 0,3 ml working solution	for 2 x 1.5 ml working solution
Lysis Tube B 2.0	10	50
Lysis Solution SLS	7 ml	35 ml
Binding Solution SBS	5 ml	20 ml
Spin Filter	10	50
Receiver Tubes	10	50
Washing Solution MS (conc.)	6 ml	24 ml
Elution Buffer	2 ml	10 ml
Manual	1	1

6.2 Components not included in the kit

- 1.5 / 2.0 ml reaction tubes
- 50 ml centrifuge tubes
- 96–98.8 % ethanol (molecular biology grade, undenatured)
- **innuPREP TCT Target Concentration Kit Beer** (845-TC-0030010/50) for liquids > 50 ml

7 Initial steps before starting

- Heat thermal mixer at 60 °C
- Add the indicated volume of ddH_2O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-KS-9000010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-9000050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated volume of absolute ethanol to **Washing Solution MS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-KS-9000010	Add 14 ml ethanol to 6 ml Washing Solution MS (conc.).
845-KS-9000050	Add 56 ml ethanol to 24 ml Washing Solution MS (conc.).

8 Product specifications

Starting material:

- Dissolved & concentrated beer sample after processing with the **innuPREP TCT Target Concentration Kit Beer** (845-TC-0030010/50) für liquids > 50 ml
- Unprocessed beer (up to 50 ml)
- Shaking culture (up to 50 ml)
- Filtrated beer (up to 50 ml) with a 45 µm filter (ø 47mm)

9 Sample preparation

9.1 Sample, concentrated with innuPREP TCT Target Concentration Kit Beer

1. Centrifuge the sample (20 ml dissolved in PBS) for 20 minutes at 5.000 x g. If the centrifuge does not allow such a high speed, use the maximum speed. Remove the supernatant as much as possible.
2. Add **600 µl Lysis Solution SLS** to the cell pellet and resuspend the pellet completely by pipetting up and down.
3. Transfer the sample into the Lysis Tube B 2.0
4. Proceed with “DNA extraction” on p. 11.

9.2 Unprocessed beer sample

1. Transfer up to 50 ml beer sample to a 50 ml tube.
2. Centrifuge the sample for 20 minutes at 5.000 x g. If the centrifuge does not allow such a high speed, use the maximum speed. Remove the supernatant as much as possible.
3. Add **600 µl Lysis Solution SLS** to the cell pellet and resuspend the pellet completely by pipetting up and down.
4. Transfer the sample into the Lysis Tube B 2.0
5. Proceed with “DNA extraction” on p. 11.

9.3 Beer shaking culture

1. Transfer up to 50 ml beer sample (beer shaking culture) to a 50 ml tube.
2. Centrifuge the sample for 20 minutes at 5.000 x g. If the centrifuge does not allow such a high speed, use the maximum speed. Remove the supernatant as much as possible.
3. Add **600 µl Lysis Solution SLS** to the cell pellet and resuspend the pellet completely by pipetting up and down.
4. Transfer the sample into the Lysis Tube B 2.0.
5. Proceed with “DNA extraction” on p. 11.

9.4 Beer filtration

1. Filtration of up to 500 ml beer depending on the turbidity of the beer sample (45 µm filter, Ø 47mm minimum)
2. Remove the filter and cut it into small pieces.
3. Put the pieces into the Lysis Tube B 2.0 and add **600 µl Lysis Solution SLS.**
3. Proceed with “DNA extraction” on p. 11.

10 DNA extraction

1. Mix Lysis Tube B 2.0 shortly by vortexing for 5 s.
2. Place the Lysis Tube B 2.0 in the Homogenizer and start the homogenization for 1 min.

NOTE
The homogenization process using commercially available homogenizers (Precellys, Fastprep, Bead Raptor etc.) can be changed and optimized depending on the used homogenizer. The optimal duration and intensity of homogenization depends on which kind of homogenizer is used.

3. Remove the Lysis Tube B 2.0 from the Homogenizer, add **50 µl Proteinase K** to the sample and vortex the tube for 5 sec.
4. Incubate the sample for 15 minutes at 60 °C using a thermoshaker.
5. Centrifuge the Lysis Tube B 2.0 at max. speed for 5 min.
6. Open the Lysis Tube B 2.0 and transfer the supernatant carefully into a new 2.0 ml reaction tube.

NOTE
Avoid carry-over of pellet-material. If the transferred supernatant contains residual pellet components, centrifuge the sample again for 2 min. at max. speed and transfer the clear supernatant into a new 2.0 ml tube.

7. Add **300 µl Binding Solution SBS** to the sample, mix by pipetting up and down several times. It is important that the sample and the Binding Solution SBS are mixed thoroughly to get a homogeneous solution.
8. Apply **750 µl of the sample** to the Spin Filter located in a 2.0 ml Receiver Tube and centrifuge at 11.000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
9. Apply the **residual sample** to the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.

10. Add **700 µl Washing Solution MS** to the Spin Filter and incubate 1 min at room temperature. Centrifuge at 11.000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the 2.0 ml Receiver Tube.
11. Add **700 µl Washing Solution MS** to the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate and reuse the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
12. Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
13. Place the Spin Filter into a 1.5 ml Elution Tube.
14. Add **100 -150 µl pre-heated Elution Buffer** (60 °C). Incubate at room temperature for 2 minutes. Centrifuge at 11.000 x g for 1 min.

NOTE
The DNA can be eluted with a lower or a higher volume of Elution Buffer (depending on the expected yield of total DNA). Store the extracted DNA at +4 °C to +8 °C. For long-time storage −22 °C to − 18 °C is recommended.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA despite a large pellet as a starting material	
Insufficient lysis	Prolong homogenization and/or lysis time.
Low concentration of extracted DNA	
Too much Elution Buffer	Elute the DNA in a lower volume of Elution Buffer.

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