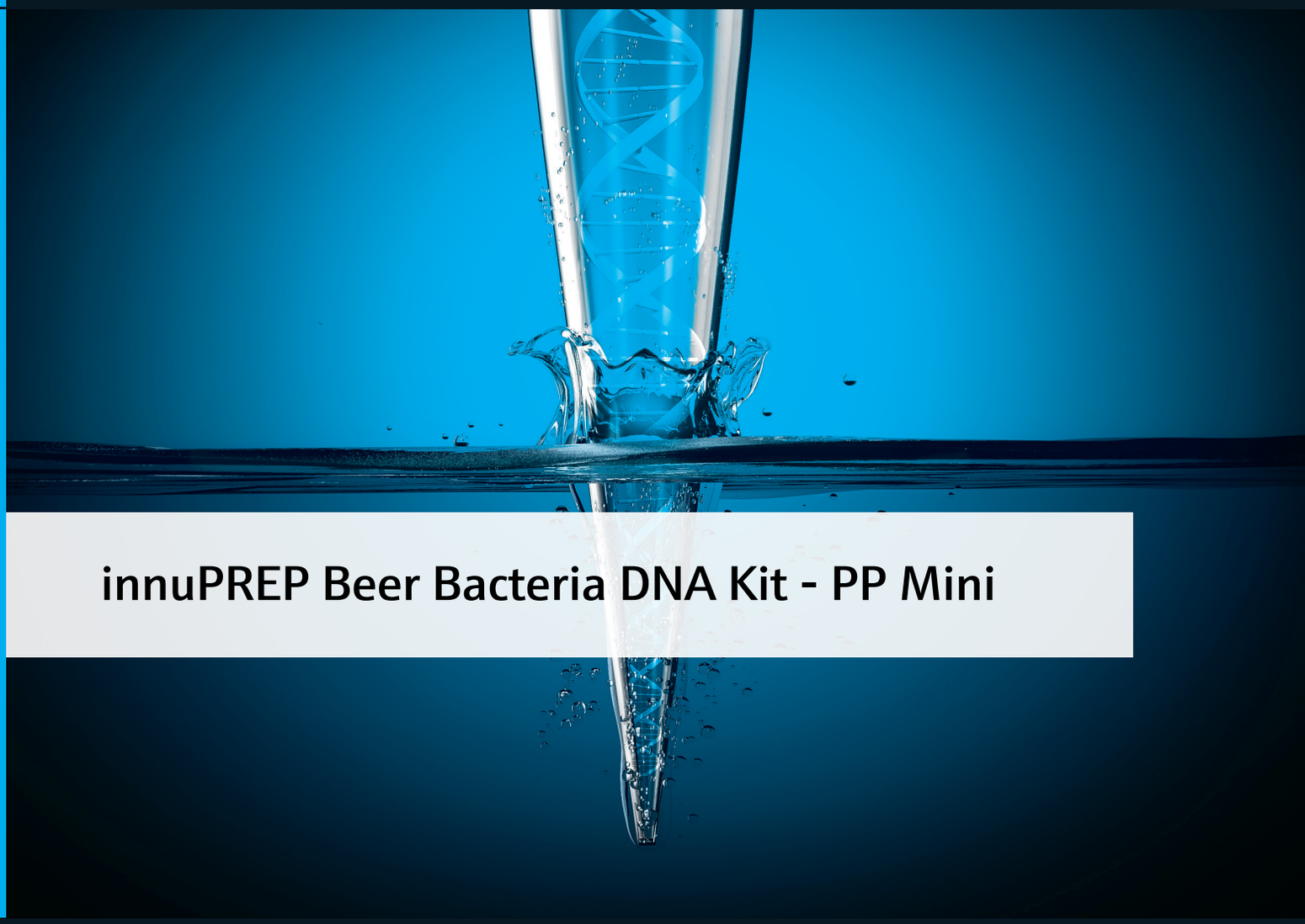
Instructions for Use
Life Science Kits & Assays



innuPREP Beer Bacteria DNA Kit - PP Mini

iST Innuscreen GmbH

Order No.:

845-PS-0010016	16 reactions
845-PS-0010096	96 reactions

Publication No.: HB_PS-0010_e_241107

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1 Introduction

1.1 Intended use

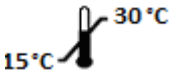
The **innuPREP Beer Bacteria DNA Kit – PP Mini** has been developed for the automated extraction of bacterial DNA from beer samples, turbid liquids and bacteria shaking cultures.
The kit utilizes a patented DNA extraction technology that integrates an initial, highly efficient homogenization step. Specially formulated buffers allow 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.

Upon arrival store **MAG Suspension F** and **Proteinase K** at 4 – 8 °C.

All other components of the 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.

If there are any precipitates within the provided solutions dissolve these precipitates by careful warming. Before every use make sure that all components have room temperature.

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 16	Σ 96
REF	845-PS-0010016	845-PS-0010096
Lysis Tube B 2.0	16	100
Lysis Solution MA	15 ml	60 ml
Proteinase K	2 x for 0.3 ml working solution	2 x for 1.5 ml working solution
Binding Solution V	10 ml	60 ml
MAG Suspension F	0.25 ml	1.1 ml
Washing Solution A	30 ml	180 ml
Washing Solution B2 (conc.)	10 ml	36 ml
Washing Solution ER	17 ml	85 ml
Elution Buffer	2 ml	15 ml
Manual	1	1

6.2 Components not included in the kit

- 50 ml centrifuge tubes
- 96–98.8 % ethanol (molecular biology grade, undenatured)
- DW Strip / DW Plate / DW Tip Comb (compatible with the PP Mini device)
- **innuPREP TCT Target Concentration Kit Beer** (845-TC-0030010/50) for liquids > 50 ml

7 Initial steps before starting

- Add the indicated volume of absolute ethanol to **Washing Solution B2 (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-PS-0010016	Add 15 ml ethanol to 10 ml Washing Solution B2 (conc.).
845-PS-0010096	Add 54 ml ethanol to 36 ml Washing Solution B2 (conc.).

- Add the indicated volume of ddH_2O to each vial of **Proteinase K** and mix thoroughly. Store as described above.

845-PS-0010016	Add 0,3 ml ddH_2O to 6 mg Proteinase K.
845-PS-0010096	Add 1,5 ml ddH_2O to 30 mg Proteinase K.

8 Product specifications

1. 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)

2. Time for automated extraction protocol on PurePrep Mini:

- Approx. 40 minutes

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 MA** 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 “Homogenization process and sample lysis” 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 MA** 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 “Homogenization process and sample lysis” 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 MA** 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 “Homogenization process and sample lysis” 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 MA.**
4. Proceed with “Homogenization process and sample lysis” on p. 11.

10 Homogenization process and sample lysis

1. Mix Lysis Tube B 2.0 shortly by vortexing for 5 s.
2. Place the Lysis Tube B 2.0 in the Thermoshaker 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 and add **20 µl Proteinase K** to the sample and vortex the sample for 5 sec.
4. Incubate the sample for 15 minutes at 60 °C using a thermoshaker.
5. Continue with 11 “Automated extraction using PurePrep Mini”

11 Automated extraction using PurePrep Mini

11.1 Prefilling of the DW Plate or the DW Strips

1. Remove the Lysis Tube B 2.0 from the Homogenizer and centrifuge the Lysis Tube B 2.0 at max. speed for 5 min.
2. Carefully transfer **the supernatant (max. 400 µl)** into **the first cavity** of the DW Strip or the DW Plate.
3. Add **10 µl** of well mixed **MAG Suspension F** and **560 µl** of **Binding Solution V**

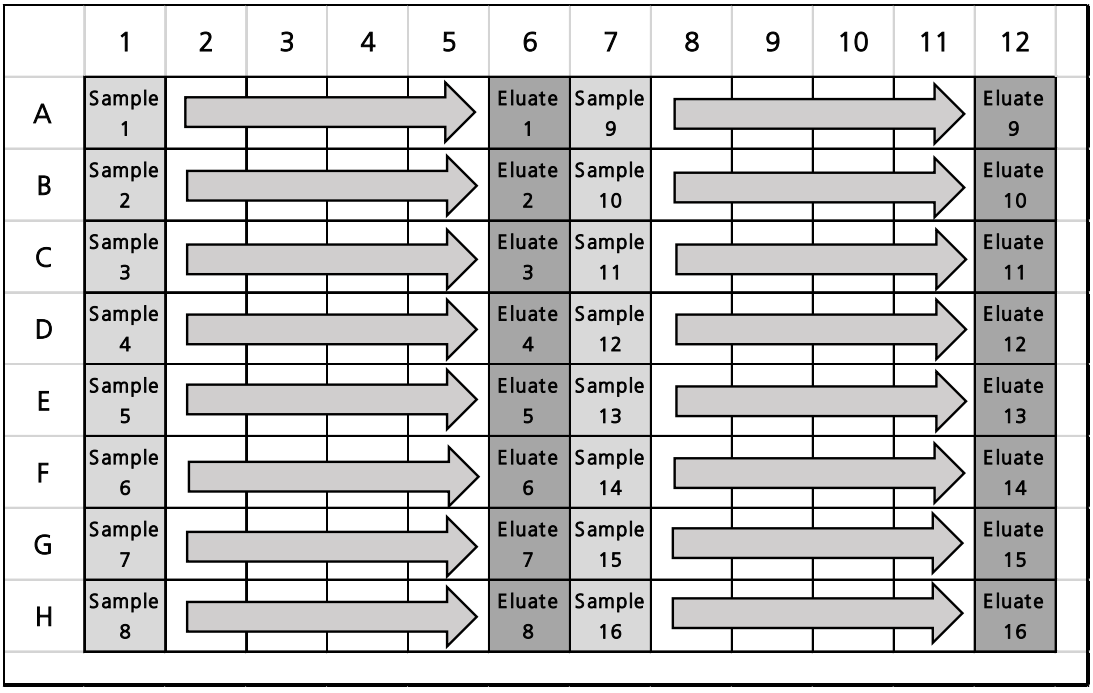


Fig. 1: Schematic illustration of DW Plate

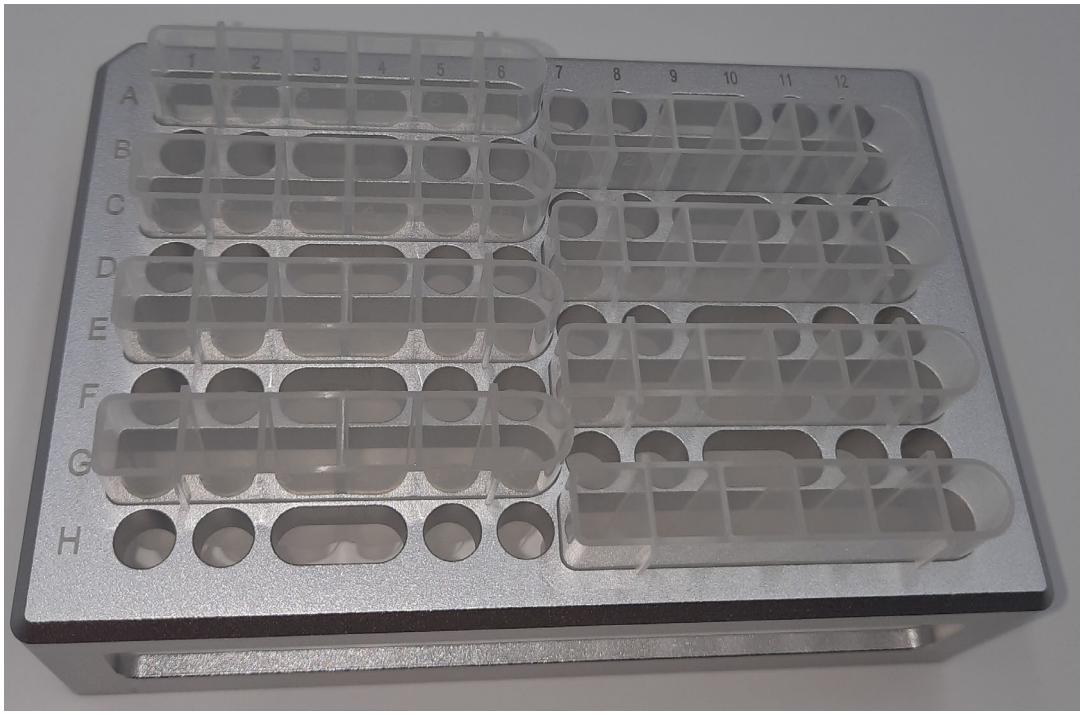


Fig. 2: Arrangement of the DW Strips in Tray

Cavity of DW Plate/Strip	Content
Cavity 1	Max. 400 µl supernatant + 10 µl MAG Suspension F + 560 µl of Binding Solution V
Cavity 2	800 µl Washing Solution A
Cavity 3	800 µl Washing Solution A
Cavity 4	800 µl Washing Solution B2
Cavity 5	800 µl Washing Solution ER
Cavity 6	100 µl Elution Buffer

11.2 Loading filled Deep Well Plate/Strips to the PurePrep Mini and plug in the Tip Combs

NOTE

- When using DW strips, the strip is inserted into the tray. In total, a maximum of 8 strips can be used in one extraction-run.
- The tip combs always dip staggered into the DW strips.

  Left tray side: Tip 1, 3, 5, 7

  Right tray side: Tip 2, 4, 6, 8.
- It is recommended to mark the tips used for the extraction so that they are not used more than once

1. Select the protocol “Beer“ and start the run.
2. After finishing the extraction protocol, the Cavity 6 & 12 contain the isolated DNA.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA despite a large pellet as a starting material	
Insufficient lysis	Prolong homogenization time.
Low concentration of extracted DNA	
Too much Elution Buffer	Elute the DNA in a lower volume of Elution Buffer (min. 80 µl).

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