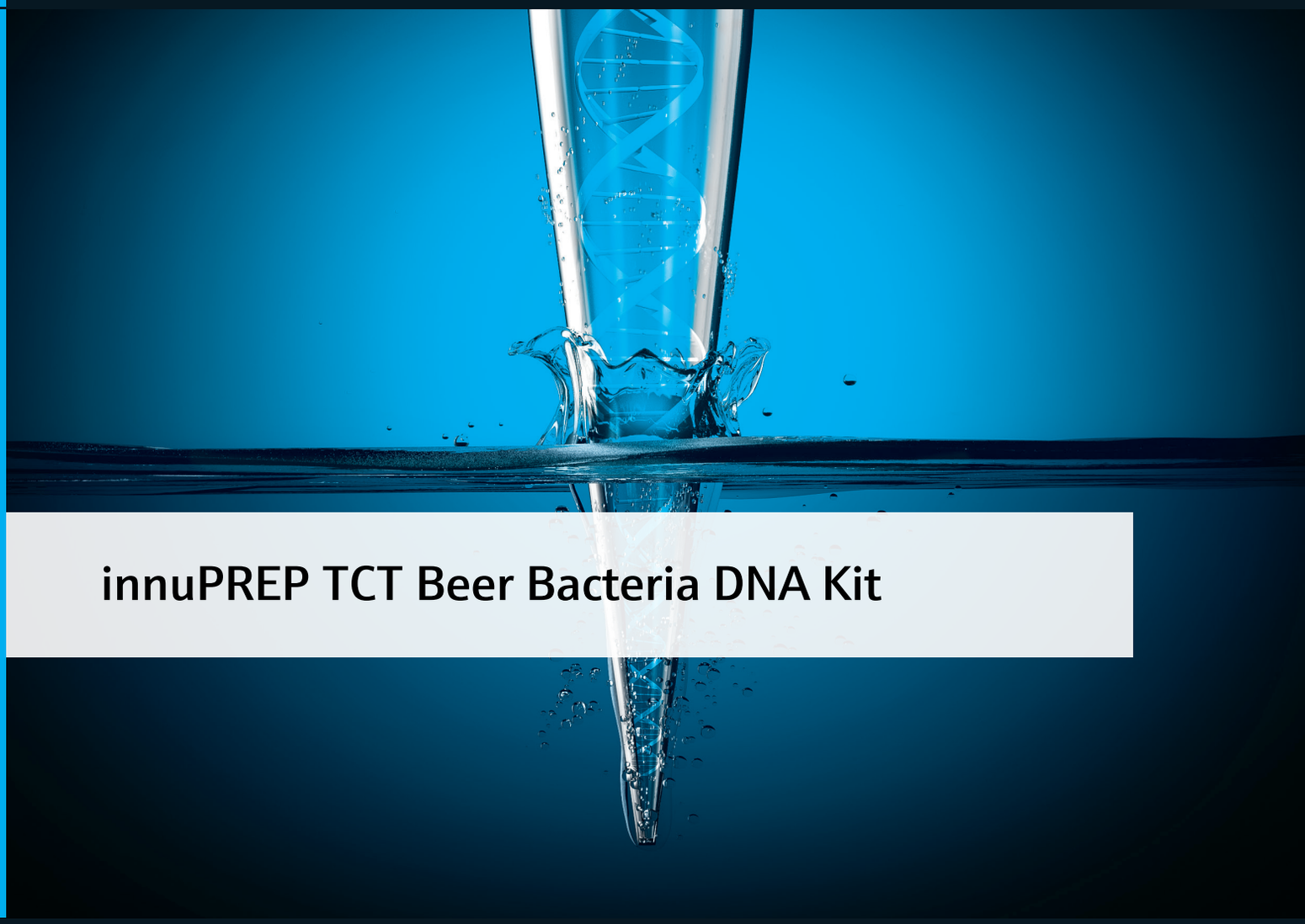
Instructions for Use
Life Science Kits & Assays



innuPREP TCT Beer Bacteria DNA Kit

Innuscreen GmbH

Order No.:
845-TC-0030010 10 reactions

Publication No.: HB_TC-0030_e_230627

Manufacturer and Distributor:
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

Contents

1 Introduction

1.1 Intended use

The **innuPREP TCT Beer Bacteria DNA Kit** uses a specially developed technique to concentrate and isolate bacterial DNA from large volume of beer samples, as well as from turbid materials and bacteria shaking cultures. There are individual isolation protocols for 100 ml beer and up to 10 ml bacteria shaking cultures.

The kit is based on a novel and patent-pending technology that allows biomolecules (cells, bacteria, viruses, bacteriophages, algae, free nucleic acids, proteins) contained in liquid samples to be concentrated and then made available for various other analysis methods. The bacterial DNA is then isolated using a specially optimized kit.

The kit is easy to handle and is divided into the following steps:

1. Target Concentration from beer samples (TCT based on native beer sample or cultivation).

2. Centrifugation of concentrated beer sample or bacterial shake culture.

3. Homogenization of bacteria sample pellet.

4. DNA extraction out of the homogenized bacteria cell pellet.

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> tests.
15 °C 30 °C	**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	**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.

All 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 10
REF	845-TC-0030010
TCT Beads (6g)	10
Lysis Tube B 2.0	10
Lysis Solution MA	9 ml
Precipitation Buffer P	2 ml
Binding Solution BL	8 ml
Spin Filter	10
Receiver Tubes	10
Washing Solution C	8 ml
Washing Solution BS (conc.)	1 ml
Elution Buffer	2 ml
Manual	1

6.2 Components not included in the kit

- 1.5 / 2.0 ml reaction tubes
- 15 ml centrifuge tubes
- 250 ml flask or another bottle for sample concentration using TCT Beads
- 96–98.8 % ethanol (molecular biology grade, undenatured)

7 Initial steps before starting

- Heat thermal mixer or water bath at 70 °C
- Add the indicated volume (9 ml) of absolute ethanol to **Washing Solution BS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-TC-0030010	Add 9 ml ethanol to 1 ml Washing Solution BS (conc.)

8 Product specifications

Starting material:

- Beer (100 ml)
- Shake culture (up to 10 ml)

9 Target concentration

9.1 Target concentration of 100 ml beer (reduction of initial volume to 5 ml – 10 ml final volume)

1. Add the **TCT Beads (6g)** into a cup or bottle with twice the volume of the initial sample.
2. Add **10 ml of ddH_20** to pre-equilibrate the **TCT Beads** and incubate at room temperature for 2 minutes.
3. Carefully add **100 ml of the beer sample** into the bottle. Avoid too much foaming of beer. Mix the sample and beads mixture carefully. Incubate at room temperature until desired target volume (5-10ml) is reached. The incubation time is approx. 60 min.

   The bottle can also be mixed briefly from time to time which speeds up the concentration somewhat.

IMPORTANT
Should it happen that the entire sample has been absorbed by the beads, only a volume of 10 ml of ddH_20 or 10 ml of the initial beer sample needs to be re-added to the beads. It is then shaken briefly, and the remaining volume is used.

4. After concentration has been completed, transfer the sample to a 15 ml tube.
5. Centrifuge the sample for 20 minutes at 2.500 x g. Remove the supernatant as much as possible.
6. Proceed with “DNA extraction from the bacteria pellet” on p. 11.

9.2 Target concentration from shaking cultures (10 to 24 h enrichment)

1. After cultivation take 10 ml of the culture and transfer it to a 15 ml tube.
2. Centrifuge the sample for 20 minutes at 2.500 x g. Remove the supernatant as much as possible.
3. Proceed with “DNA extraction from the bacteria pellet” on p. 11.

10 DNA extraction from the bacteria pellet

10.1 Homogenization process

1. Add **650 µl Lysis Solution MA** to the cell pellet and resuspend the pellet completely by pipetting up and down.
2. Transfer the sample into the Lysis Tube B 2.0 and mix shortly by vortexing for 5 s.
3. 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 (SpeedMill, 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.

10.2 DNA Extraction

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. Open the Lysis Tube B 2.0 and transfer the supernatant carefully into a new 2.0 ml reaction tube. Add **50 µl Precipitation Buffer P** and vortex shortly for 5 s. Leave the tube for 2 min.
3. Centrifuge at maximum speed for 5 min. Carefully transfer the supernatant into a new 2.0 ml tube (determine the volume of the supernatant).

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.

4. Add an equal volume **Binding Solution BL** to the sample, mix by pipetting up and down several times. It is important that the sample and the Binding Solution BL are mixed thoroughly to get a homogeneous solution.
5. 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.
6. 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.
7. Add **700 µl Washing Solution C** 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.
8. Add **700 µl Washing Solution BS** 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.
9. Add **400 µl absolute ethanol** to the Spin Filter and centrifuge at max. speed for 3 minutes to remove all traces of ethanol. Carefully remove the spin filter and place the Spin Filter in a 1.5 ml tube. Discard the 2.0 ml Receiver Tube.
10. Add **50 - 100 µl pre-heated Elution Buffer** (70°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.
In rare cases, the eluate may be discolored. If the eluate is discolored, it is recommended to use the cleanup protocol.

11 Troubleshooting

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

IST Innuscreen GmbH
Robert-Rössle-Str.10
13125 Berlin · Germany

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

IST Innuscreen GmbH