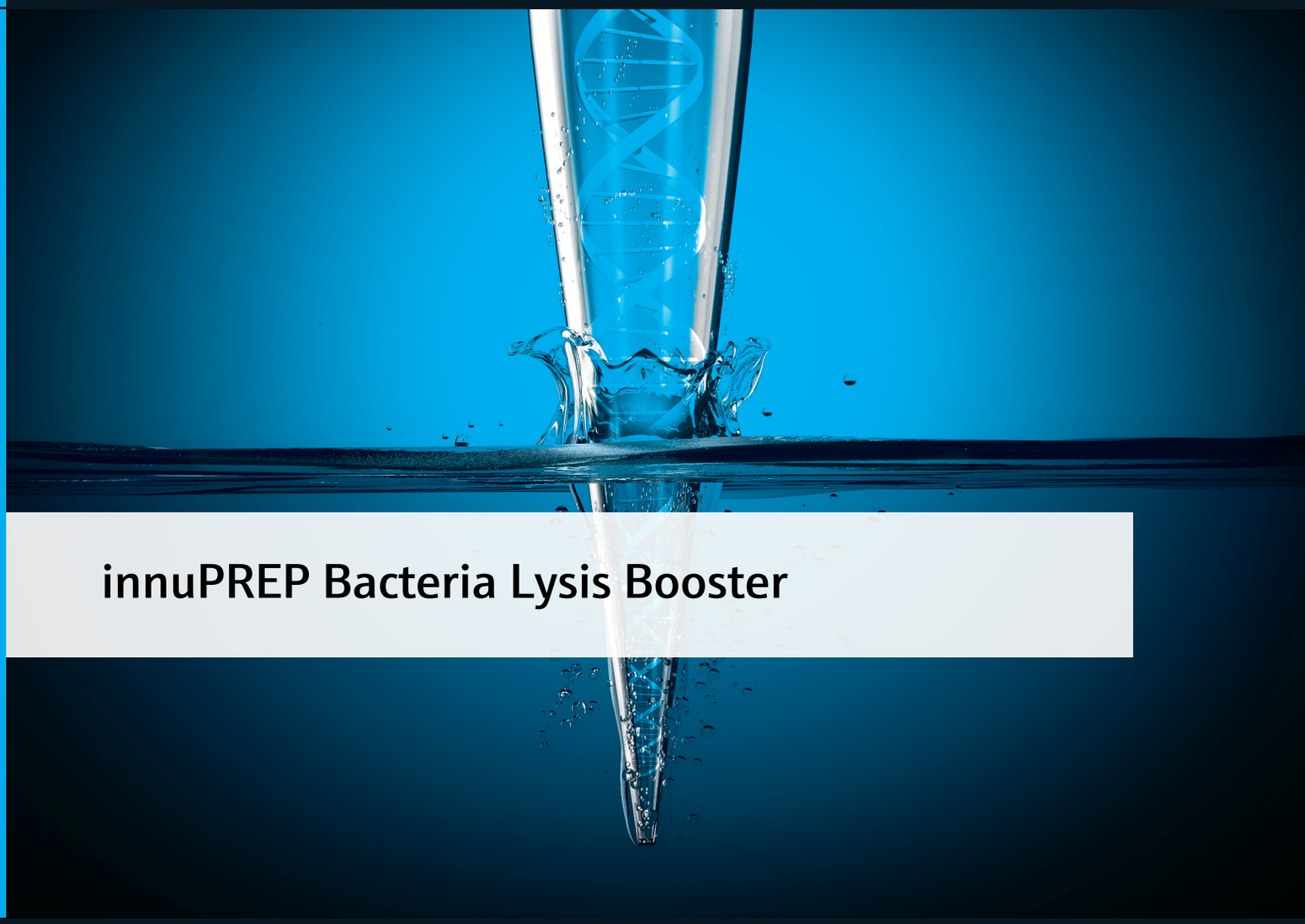
Instructions for Use
Life Science Kits & Assays



innuPREP Bacteria Lysis Booster

Your distributor:
westburg LIFE SCIENCES
Arnhemseweg 87 | 3832 GK Leusden | The Netherlands
westburg.eu | info@westburg.eu | +31(0)33 495 00 94

iST Innuscreen GmbH

1 Product specifications

1. Starting material:
   - Up to 2 x 10^9 of bacteria cells
   - Gram-positive or Gram-negative bacteria
2. Time for lysis:
   - approx. 30 minutes
3. Typical yield:
   - Depending on amount of starting material and condition of bacterial cells.
   - The yield of isolated DNA, RNA and proteins is affected by the concentration of starting material and by the extraction kit used.

2 Intended use

The innuPREP Bacteria Lysis Booster has been developed for a highly efficient non-mechanical 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 spp*., *Lactobacillus spp*., *Staphylococcus spp*., *Bacillus spp*. and *Clostridium spp*..

The resulting spheroblasts can be used in all lysis systems for isolation of DNA, RNA. The best results will be obtained by usage of the kits from IST Innuscreen GmbH.

In combination with the newly introduced SmartExtraction technology by IST Innuscreen GmbH the obtained DNA is not only of outstanding yield and quality but also high molecular and therefore well suited for size sensitive downstream applications.

For research only!

3 Product and order number

Name	Amount	Order-no.
innuPREP Bacteria Lysis Booster	50 rxn	845-KA-1000050

4 Storage conditions

The innuPREP Bacteria Lysis Booster is shipped at ambient temperature.

Upon receipt store the kit at -22 °C to -18 °C.

When stored as recommended, the kit is stable until the expiration date printed on the label on the kit box.

Store prepared Enzyme Mixes at -22 °C to -18 °C for a maximum of 3 weeks.

5 Safety precautions

The kit shall only be handled by educated personnel in a laboratory environment. The compliance with the specified procedure is absolutely mandatory when performing this assay.

Reagents should be stored in their original containers at the indicated temperatures. Do not replace individual components with those from different batches or kits. Note the indicated expiration dates.

Do not eat, drink or smoke while performing the assay.

Wear protective clothing and safety gloves.

All samples and test materials should be handled and disposed of as infectious material, in accordance with regulatory requirements.

Reagent containers that have not come in contact with potentially infectious material may be disposed of along with ordinary laboratory waste.

6 Delivered components

Components	Σ 50
Enzyme A	1 vial, lyophilized
Enzyme B	300 µl
Enzyme C	300 µl
Storage Buffer A	600 µl
TE Buffer	15 ml

NOTE

The innuPREP Bacteria Lysis Booster provides spheroblasts. The kit does not perform a complete lysis of bacterial cells, living bacteria could be still present in the sample. The sample should be handled after regional safety regulations.

7 Initial Steps before starting

- **Enzyme A**:

  Dissolve Enzyme A by addition of **550 µl** of **Storage Buffer A**, mix thoroughly and store as described above.

- Prepare the lysis mix after the according to table below.

Number of reactions	Enzyme A	Enzyme B	Enzyme C
1 x	11 µl	5.5 µl	5.5 µl
2 x	22 µl	11 µl	11 µl
3 x	33 µl	16.5 µl	16.5 µl
16 x	176 µl	88 µl	88 µl
n reactions	11 x n µl	5.5 x n µl	5.5 x n µl

8 Protocols

8.1 Lysis of bacteria from liquid culture

1. Transfer the bacterial culture (volume depends on the concentration of starting material) into a 2 ml tube (e.g. 2 ml of a 1 x 10^9 culture)
2. Centrifuge 10 minutes at 3,000 x g to pellet the bacterial cells.
3. Carefully discard the supernatant by pipetting. Do not discard the pellet!
4. Add **170 µl TE-Buffer** and resuspend the pellet carefully.
5. Vortex the **Enzyme M**ix (prepared as described above) thoroughly.
6. Add **20 µl** of the **Enzyme Mix**. Vortex the sample shortly.
7. Incubate sample for 30 minutes at 37 °C and 550 rpm in a shaking platform.
8. Sample preparation completed. Proceed with your specific purification protocol.

8.2 Protocol for Isolation of DNA/RNA/proteins from colonies from agar plates

1. Add **170 µl TE-Buffer** to a 2.0 ml tube.
2. Pick up the colonies from the plate with a sterile inoculation loop.
3. Transfer the colonies to the tube with **TE-Buffer**.
4. Vortex the prepared Enzyme Mix thoroughly.
5. Add **20 µl** of the **Enzyme Mix**. Vortex the sample shortly.
6. Incubate sample for 30 minutes at 37°C and 550 rpm in a shaking platform.
7. Sample preparation completed. Proceed with your specific purification protocol.

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Manufacturer & Distribution:
IST Innuscreen GmbH
Robert-Rössle-Strasse 10
13125 Berlin · Germany

Telefon +49 30 9489 3380
Telefax +49 30 9489 3381
info.innu@ist-ag.com