Lysostaphin

Lysostaphin is a recombinant enzyme which has an ability to hydrolyze the cell walls of bacteria from a *Staphylococcus* genus. It is an endopeptidase which specifically cleaves the pentaglycine cross bridges found in the staphylococcal peptidoglycan. *Staphylococcus aureus* cell walls contain high proportions of pentaglycine, making lysostaphin a highly effective agent against both actively growing and quiescent bacteria. It has also been shown to be effective against *S. epidermidis*, but at higher concentrations of the enzyme. The specificity of the pentaglycine peptide is very high and other gram–positive and gram–negative bacteria are not susceptible to this enzyme. The unique biological activity of lysostaphin presents numerous possibilities for its application in the medical, veterinary, food industry and research fields. It is a major component of most commercially available kits for the extraction of DNA from staphylococci.

The lysostaphin enzyme can be used directly with the *EXTRACTME DNA BACTERIA kit* (EM02) when isolating DNA from the *Staphylococci*. 
Features

- Specific activity: 400 U/mL
- Concentration: 1 mg/mL
- Origin: recombinant lysostaphin from *Staphylococcus simulans* expressed in *E. coli*
- Purity: >95% (SDS-PAGE)
**Lysostaphin**

**Applications**

- Lysostaphin is widely used as a research and diagnostic tool when preparing staphylococcal DNA or other cellular components for genetic and biochemical studies and for the preparation of protoplasts for transformation.
- *In vitro* and *in vivo* studies performed with lysostaphin have shown that this enzyme has a potential use, either alone or in combination with other antibacterial agents, in the prevention or treatment of infectious bacterial staphylococcal diseases.
- There are also examples of the application of this enzyme in food protection.
Protocol

The following protocol should be followed when isolating DNA from the *Staphylococci* with the *EXTRACTME DNA BACTERIA* kit (EM02). When performing isolation using other extraction kits, follow the protocol of the manufacturer’s kit.

1. Pellet cells by centrifugation of 1.5 ml of bacterial culture\(^1\).
2. Discard the supernatant and suspend the cell pellet in 200 μl TE\(^2\). Mix thoroughly.
3. Add 30 μl lysostaphin 400 U/ml solution and 4 μl RNase A. Mix well by pipetting or vortexing.
4. Incubate at 37°C for 20-60 min\(^3\) (until sample is lysed).
5. Add 300 μl BacL Buffer and 17 μl Proteinase K. Mix thoroughly.
6. Continue the isolation following the Isolation Protocol from step 6 (section XI).

1) For thick cultures, use less cell culture.
2) TE Buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.
3) When isolating from coagulase-negative strains, use 50 μl of lysostaphin and incubate 1h at 37°C.
Additional considerations

→ The best results can be obtained when using lysostaphin to the final reaction concentration of 50–80 U/ml (for example: use 30 µl lysostaphin 400 U/ml in 200 µl TE buffer).
→ The protocol for use of the lysostaphin can be found in the instruction manual of *EXTRACTME DNA BACTERIA* kit.
→ When performing isolation using other extraction kits, follow the protocol of the manufacturer’s kit.

Storage buffer

10 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 50% (v/v) glycerol
Quality control

Lysostaphin is functionally tested in *S. aureus* DNA extraction assays and is free of detectable contaminating nuclease activities.

Unit definition

One unit is defined as an amount of enzyme required to reduce the turbidity ($A_{620}$) of a suspension of *Staphylococcus aureus* cells from 0.25 to 0.125 in 10 min at pH 7.5 at 37°C in a 6 ml reaction mixture.
Lysostaphin

<table>
<thead>
<tr>
<th>Component</th>
<th>RP12 400 U</th>
<th>RP125 2000 U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysostaphin 400 U/ml</td>
<td>1 ml</td>
<td>5 x 1 ml</td>
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</tbody>
</table>

Storage & shipping

**Storage conditions**
Lysostaphin should be stored at -20°C. The enzyme remains stable for at least 12 months from the date of purchase providing it is stored properly.

**Shipping conditions**
Shipping on dry or blue ice.

Date of purchase  
Warranty  
12 months from the date of purchase