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Product Information

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A3233 - Lysozyme

Source: Chicken egg white
Activity: >20,000 units/mg
CAS number: 12650-88-3

Description

Lysozyme is a single chain polypeptide of 129 amino acids cross-linked with four disulfide bridges. It inhibits the biological activity of lipopolysaccharides from periodontopathic bacteria.

It hydrolyzes ß1, 4 linkages between N-acetylmuraminic acid and N-acetyl-D-glucosamine residues in peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrin. It also hydrolyzes β1, 4 linkages between N-acetylmuramic acid and N-acetyl-glucosamine residues in peptidoglycans.

Unit Definition

One Shugar unit is defined as the amount of enzyme that will cause a decrease in absorbance of 0.001 at 450 nm per min due to lysis of Micrococcus luteus at 25°C, pH 6.2.

Solubility

It dissolves readily at 10mg/ml in water or 10 mM Tris-HCl, pH 8.0.

Storage/Stability

The product, as supplied, should be stored at –20 °C. When stored at –20 °C, the enzyme retains activity for at least 4 years. Solutions (pH 4–5) remain active for several weeks if refrigerated.

Molecular Weight: 14,388
Isoelectric point (pI): 11.35
Extinction coefficients:

- E1%(281.5 nm): 26.4 in 0.1M potassium chloride
- EmM(280 nm): 36

Optimal pH: The activity of lysozyme is a function of both pH and ionic strength. The enzyme is active over a broad pH range (6.0–9.0). At pH 6.2, maximal activity is observed over a wider range of ionic strengths (0.02–0.100M) than at pH 9.2 (0.01–0.06 M).

Inhibitors:

Lysozyme is inhibited by indole derivatives, which bind to and distort the active site, and imidazole, which induces the formation of a charge-transfer complex. It is also inhibited by surface-active agents such as sodium dodecyl sulfate, sodium dodecanate, and dodecyl alcohol. Other compounds of these types with carbon chains of 12 or more carbons in length will also inhibit lysozyme.

Substrates:

The natural substrate for lysozyme is the peptidoglycan layer of bacterial cell walls. However, a variety of low molecular mass substrates including murein degradation products as well as synthetic compounds have been used for various photometric, isotopic, and immunological lysozyme assays.

The following low molecular mass lysozyme substrates are available:

- 4-Methylumbelliferyl b-D-N,N¢,N¢¢-triacetyl-chitotrioside (a fluorogenic substrate)
- 4-Nitrophenyl b-D-N,N¢,N¢¢-triacetylchitotriose.

Preparation Instructions

The following procedure is for the lysis of E. coli. It may be used as a guideline for other species.
The optimal pH for E. coli cell lysis is 8.0±0.1.9.
Use a freshly prepared lysozyme solution (10 mg/ml) in 10 mM Tris-HCl, pH 8.0.

1. Incubate E. coli bearing the pBR322 plasmid overnight in Terrific Broth with 25 mg/ml tetracycline and 25 mg/ml ampicillin.
2. Centrifuge 1–2 ml samples of the overnight culture.
3. Resuspend the pellets in 350 ml of STET buffer (10 mM Tris-HCl, pH 8.0, with 0.1 M NaCl, 1 mM EDTA, and 5% [w/v] TRITON X-100).
4. Add 25 ml of a freshly prepared lysozyme solution (10 mg/ml in 10 mM Tris-HCl, pH 8.0).
5. Mix by vortexing for 3 seconds.
6. Incubate the lysis mixture for 30 minutes at 37 °C.
7. After incubation, place the tube containing the lysis mixture in a boiling water bath for exactly 40 seconds.
8. Centrifuge the lysis mixture at 14,000xg.
9. Remove the pellet (cell debris) from the tube using a sterile toothpick.
10. Plasmid DNA from the supernatant may then be purified and analyzed.