Lysozyme

Product No.: 101-12650-88-3

Description

Lysozyme is an enzyme characterized by the ability to break down the bacterial cell wall to improve protein or nucleic acid extraction efficiency. Lysozyme is an enzyme used to break down bacterial cell walls to improve protein or nucleic acid extraction efficiency. Lysozymes (muramidases) are a family of enzymes with antimicrobial activity characterized by the ability to damage the cell wall of bacteria. The enzyme acts by catalyzing the hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycans and between the N-acetyl-D-glucosamine residues in chitodextrins. Although hen egg white lysozyme is most effective for the lysis of gram-positive bacteria, it also facilitates the lysis of gram-negative bacteria such as Salmonella and Shigella. The lysis of E. coli is especially improved by the addition of both lysozyme and a nuclease such as DNase I.

Structure

Lysozyme consists of a single chain polypeptide containing 129 amino acid residues which is cross-linked with 4 disulfide bridges. Its molecular weight of is 14,307 based upon amino acid sequence. Lysozyme possesses a binding site for a hexasaccharide segment of peptidoglycan. The substrate is thought to bind in a cleft located between two halves of the enzyme molecule. The enzyme promotes catalysis by inducing steric strain in the substrate. The presence of Asp52 and Glu35 on either side of the substrate cleavage site aids in catalysis

Unit Definition

The amount of enzyme causing a decrease in absorbance at 450nm of 0.001 per minute at 25 ºC and pH 6.2 with Micrococcous Lysodeikticus as substrate. Hydrolyses the beta-1,4-glycosidic binding between N-Acetyl muraminic acid and N-Acetyl glucosamine, a component of the proteoglycan-cell wall of certain microorganism. The enzyme is present in many organisms. In molecular biology, the enzyme from chicken white egg is used to lyse E. coli for the isolation of plasmid-DNA. Another application is the lysis of bacteria for the preparation of bacterial RNA.

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 (0.02–0.100 M) than at pH 9.2 (0.01–0.06 M).

Specification

Recommended Usage: Molecular Biology Reagent
DNA/RNA Experiment, For DNA/RNA Isolation
Physical State: Solid

Appearance: White to off-white crystalline powder
Color: White
Odor: Odorless
Molecular Mass: 14,307 Da (amino acid sequence). The molecular weight is 14,307 based upon amino acid sequence and 14,400 by sedimentation equilibrium
Activity (25 ºC): >20,000 U/mg

Application

1. Hydrolysis of bacterial cell walls: Lysozyme is widely used in the enzymatic lysis of microbial cells. It hydrolyzes the β-1,4 glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in the polysaccharide backbone of peptidoglycan present in bacterial cell walls. Gram-positive bacterial cell walls contain a high proportion of peptidoglycan and are quite susceptible to hydrolysis by lysozyme. Gram-negative bacteria are less susceptible to hydrolysis since they have a lower proportion of peptidoglycan and an outer membrane. They may be made more susceptible to lysis by the addition of EDTA, which chelates metal ions in the outer bacterial membrane. This optimizes the lysis of the bacterial cell wall with lysozyme.
2. Hydrolysis of chitin: Lysozyme will also hydrolyze chitin oligosaccharides.
3. Nucleic acid preparation: DNA/RNA Experiment, For DNA/RNA Isolation
4. Plasmid preparation (to break down membranes and cell wall): It is suitability tested as a lysing agent in the purification of plasmid DNA from E. coli.
5. Protein purification from inclusion bodies

Preparation Instructions

For E. coli cell lysis, use a freshly prepared lysozyme solution (10 mg/ml) in 10 mM Tris-HCl, pH 8.0. The product is also soluble in water (10 mg/ml) yielding a clear to slightly hazy colorless solution. Aqueous solutions should retain activity for at least one month when stored between 2–8 ºC.

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. Lysozyme is also inhibited by N-acetylglucosamine (NAG) and lactone analogs of peptidoglycan.

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 β-D-N,N,N-triacetyl chitotrioside ; 4-Nitrophenyl β-D-N,N,N-triacetylchitotriose

Recommended Procedures for the Protein Extraction

Below is a recommended protocol for the extraction of proteins from E. coli using this lysozyme solution. It may be used as a guideline for other species. Addition of nucleases, such as benzonase, may help reduce the viscosity of the released chromosomal DNA. Protease inhibitors may also be added to prevent breakdown of proteins during cell lysis (Protease Inhibitor Cocktail for Poly-His proteins).

1. Collect the cells that express the protein of interest by centrifuging at 5,000 x g for 10 minutes.
2. Carefully remove the media from the cell pellet. The cell pellet may be frozen or used fresh.
3. Use 10 ml of CelLytic plus 0.2 ml of lysozyme solution (final concentration of 0.2 mg lysozyme / ml) per gram of cell paste. Mix the sample well to completely resuspend the cells.
4. Incubate the extraction suspension with shaking at room temperature for 10-15 minutes to fully extract the protein from the cells.
5. Centrifuge the extract at 1,900 x g for 15 minutes to pellet the insoluble material. For very viscous extracts, centrifuge at 25,000 x g for 15 minutes.
6. Carefully remove the supernatant containing the soluble proteins. Approximately 90 to 95% of the soluble proteins will be found in this fraction.

Storage / Stability: Store at -20 ºC. Protect from moisture.