ProductInformation

LYSOZYME GRADE I
from Chicken Egg White
Molecular Biology Reagent
Sigma Prod. No. L7651

CAS Number: 12650-88-3
ENZYME COMMISSION NUMBER: 3.2.1.17

SYNONYMS: Muramidase, Peptidoglycan N-acetylmuramoylhydrolase

PHYSICAL DESCRIPTION:

Appearance: White to off-white powder
Molecular weight: The molecular weight is 14,307 based upon amino acid sequence and 14,400 by sedimentation equilibrium.[1,2]
$E^{1\%}$(281.5 nm) = 26.4 in 0.1 M potassium chloride.[3] An EmM (280 nm) = 36 has also been reported in the literature.[5]
Isoelectric point: The pI of lysozyme is 11.35.[4]
pH Optimum: The activity of lysozyme is a function of both pH and ionic strength. Lysozyme 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) than at pH 9.2 (0.01-0.06).[5] Sigma determines the activity of this product at pH 6.24. Lysis of E. coli by lysozyme is conducted at pH 8.0[6]
Salts present: The salt content of this product is approximately 5% which is present as sodium acetate and sodium chloride buffer salts.

STRUCTURE:

Lysozyme consists of a single chain polypeptide containing 129 amino acid residues which is cross-linked with 4 disulfide bridges.[7] 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.[8,9]

INHIBITORS:

Lysozyme is inhibited by indole derivatives (which bind to and distort the active site) and imidazole (formation of a charge-transfer complex).[10] It is also inhibited by surface-active agents such as sodium dodecyl sulfate, sodium dodecanate, and dodecyl alcohol. Other compounds of these types will inhibit lysozyme provided that the carbon chain present is at least 12 or more carbons in length.[11] Lysozyme is also inhibited by N-acetylglucosamine (NAG) and lactone analogs of peptidoglycan.[9]

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SUBSTRATES:

Lysozyme hydrolyzes the B(164) glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in the polysaccharide backbone of peptidoglycan. It is effective in lysing bacteria by hydrolyzing the peptidoglycan which is present in bacterial cell walls. The substrate used in the Sigma enzyme assay for this product is Micrococcus luteus cells (ATCC 4698). Lysozyme will also hydrolyze chitin oligosaccharides.[12,13]

APPLICATIONS:

Lysozyme is widely used in the enzymatic lysis of microbial cells. 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 since they have a lower proportion of peptidoglycan and there is also outer membrane present. They may be made more susceptible to lysis by the addition of EDTA. EDTA chelates metal ions in the outer bacterial membrane, which optimizes the lysis of the bacterial cell wall with lysozyme.[14] Each lot number of this product is suitability tested as a lysing agent in the purification of plasmid DNA from E. coli. The protocol is as follows: E. coli strain (ATCC: 37017) cells bearing the pBR322 plasmid were incubated overnight in Terrific broth (T-0918) with 25 µg/ml Tetracycline (T-3383) and 25 µg/ml Ampicillin (A-9518). 1-2 ml samples of the overnight culture were centrifuged and cell pellets were resuspended in 350 µl of STET buffer (10 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 1 mM EDTA, 5% w/v Triton X-100). 25 µl of a freshly prepared solution of lysozyme (500,000 µn/ml in 10 mM Tris HCl, pH 8.0) was then added and mixed by vortexing for 3 seconds. The lysis mixture was incubated 30 minutes at 37°C. and placed in a boiling water bath for exactly 40 seconds. After centrifuging the lysis mixture at 14,000 x g, the pellet (cell debris) was removed with a sterile toothpick. Plasmid DNA from the supernatant was then purified using NucleiClean™ Nucleic Acid Rapid Isolation Kit (Stock No. D-RAP) and analyzed by agarose electrophoresis. Lysozyme treated cells released >30 µg pBR322 DNA. The enzymatic lysis of Echerichia coli by lysozyme is also described.[15]

METHOD OF PREPARATION:

A method of preparation for lysozyme (although not that necessarily used by Sigma) is described.[16,17]

STABILITY/STORAGE AS SUPPLIED:

This product is stable for up to 4 years when stored in a freezer.

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SOLUBILITY / SOLUTION STABILITY:

When this product is solubilized at 10 mg/ml in deionized water, a clear to slightly hazy colorless solution is observed. Solutions prepared in this way should be stable for at least 1 month when stored at 2-8°C.

UNIT DEFINITION:

One unit will produce a ΔA_{450nm} of 0.001 per min at pH 6.24 at 25°C., using a suspension of Micrococcus lysodeikticus as substrate in a 2.6 ml reaction mixture (1 cm light path).

REFERENCES:

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