vivantis

PRODUCT INFORMATION
Lysozyme

Product: Lysozyme

Grade: Molecular Biology Grade

Code: PC0710-1g / 5g / 10g

Molecular Mass: 14307 Da (amino acid sequence)

Lot No.:

Expiry Date:

Description

Lysozyme is a single chain polypeptide of 129 amino acids cross-linked with four disulfide bridges. It hydrolyzes β(1-4) linkages between N-acetylmuraminic acid and N-acetyl-D-glucosamine residues in peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrin. Lysozyme is used for bacterial cells lysis by hydrolyzing the peptidoglycan in cell walls. Gram-positive bacteria cells which have a high proportion of peptidoglycan are quite susceptible to the hydrolysis while gram-negative bacteria cells which have lower proportion of peptidoglycan as well as have the presence of outer membrane are less susceptible to the hydrolysis. With the presence of EDTA that chelates metal ions in the outer bacterial membrane, these cells may be hydrolyzed more easily.

The lysozyme is supplied in lyophilized powder form. It is being purified from chicken egg white and being crystallized and dialyzed into lyophilized form. The highly purified enzyme preparation has been used in mass spectrometry as a protein mass calibration standard and in structural studies of protein. It is suitable to be used in plasmid DNA purification as a lysing agent in a boiling lysing technique.

Isoelectric point (pI): 11.35

Extinction co-efficients: $E^{1\%}$ (281.5nm): 26.4 in 0.1M potassium chloride

E^{mM} (280nM): 36

Lysozyme is active between pH 6.0 – pH 9.0. At pH 6.2, maximal activity is observed over a wider range of ionic strengths (0.02-0.10M) than pH 9.2 (0.01-0.06M).

Unit Definition

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

Lysozyme activity: ≥20,000 units/mg protein

Storage Temperature:

Store at -20°C. Avoid exposure to frequent temperature changes. See the expiration date on the stickers of product item. Solutions (pH 4-5) remain active for several weeks if refrigerated. Recommend to always prepare fresh.

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. 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-Methylumbeliferyl β-D-N,N',N''-triacetyl-chitotrioside (a fluorogenic substrate)
- 4-Nitrophenyl β-D-N,N',N''-triacetylchitotriose

Preparation Instructions

The suggested concentration of lysozyme is **10mg/ml**.
The suggested volume for bacterial cell lysis:

- **Gram positive** bacterial cell: **50-100µl of 10mg/ml lysozyme solution in 1-3ml bacterial pellet**
- **Gram negative** bacterial cell: **10-50µl of 10mg/ml lysozyme solution in 1-3ml bacterial pellet**

The reagents used to reconstitute the lyophilized lysozyme:

- **10mM Tris-HCl, pH8.0** (The aqueous solution should retain activity for at least one month when stored between 2-8°C or at least three month when stored at -20°C)
- **Sterile water** (Yielding clear to slightly hazy colorless solution. The lysozyme solution that prepared by water can be freshly used, not recommend to keep the stock as the solution cannot retain activity.)

Suggested Procedure

1. Incubate *E.coli* bacteria overnight in LB Broth with 25µg/ml of antibiotic.
2. Centrifuge 1-2ml samples of the overnight culture.
3. Resuspend the pellets in 350µl of STET buffer or the volume of lysis buffer that suggested by commercial kits.
4. Add 25µl of a freshly prepared lysozyme solution (10mg/ml in 10mM Tris-HCl, pH8.0).
5. Mix by vortexing for 3 seconds.
6. Incubate the lysis mixture at 37°C for 30 minutes.
7. After incubation, follow the steps suggested by commercial kits. Or
   After incubation, place the tube containing the lysis mixture in a boiling water bath for 40 seconds.
8. Centrifuge the lysis mixture and remove the pellet (cell debris) from the tube using a sterile toothpick.
9. Bacterial DNA from the supernatant may then be purified and analyzed.

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