# Lysozyme

**Cat# ER1004 – 10 g**

**Storage at -20 ℃ and protect from moisture**

## INTRODUCTION

**Lysozyme** is an enzyme used to break down bacterial cell walls to improve protein or nucleic acid extraction efficiency. 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 nucleases such as DNase I.

## INFORMATION

**Synonyms**: Muramidase; Lysozyme c; Mucopeptide N-acetylmuramoylhydrolase

**Recommended Usage**: 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.

**Purity/Assay**: 98% Min

**Activity (25℃)**: >23,500U/mg

## UNIT DEFINITION

The amount of enzyme causing a decrease in absorbance at 450nm of 0.001 per minute at 25℃ 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.

## 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**

## PROTOCOL

**1. Preparation**

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 ℃.

> **Note** : 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).

**2. Procedure 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 Cell Lytic 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.

## PRODUCT USE LIMITATION

These products are intended for research use only.