

# Lysozyme Kit

HAMPTON  
RESEARCH

Solutions for Crystal Growth

## User Guide

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### Description

The Lysozyme Kit contains 12 vials with 20 milligrams of lysozyme and 12 vials of 1 milliliter of 0.02 M Sodium acetate trihydrate pH 4.6 (solubilization buffer). The kit allows for the neat, rapid and convenient formulation of a lysozyme working solution for crystallization demonstrations, teaching, and validation of instrumentation. To use, simply pipet the solubilization buffer into one of the vials containing lysozyme. Gently rock the vial to solubilize the lysozyme at room temperature. Shaking, vortexing, and violent confrontations with the vial and solution can denature the lysozyme and reduce the chances for crystallization to nil. One may vary the volume of solubilization buffer added to the lysozyme to create different working concentration of lysozyme (Table 1).

Table 1

Microliters of Solubilization Buffer Added	Final Lysozyme Concentration
1,000	20 mg/ml
800	25 mg/ml
500	40 mg/ml
400	50 mg/ml
200	100 mg/ml

One may use the formulated lysozyme solution without centrifugation or filtration. Or, one may centrifuge the sample to sediment amorphous non soluble material. Or, one may filter the sample using a 0.22 or 0.45 micron pore size filter. Typically, one observes less crystal nucleation with centrifuged or filtered samples. Once formulated, the sample may be stored at 25°C for 24 hours, 4°C for up to 30 days, and frozen at -20 to -70°C for up to one year.

Product Number	HR7-108
Lot Number	710816
Description	Lysozyme, lyophilized
Synonym	Mucopeptide-N-acetylmuramoylhydrolase
Source	Chicken egg white
Appearance	Lyophilized, white to off-white crystalline powder 3x crystallized
Storage	4°C or below. Protect against moisture.
Optimum pH	9.2 (Davies et al. 1969)
Analysis	Result
Ash (Residue Upon Ignition)	Maximum 1.0%
Moisture (Loss on Drying)	Maximum 6.0%
pH Range	3.0 - 4.5

Conductivity Range	2014 $\mu$ S/cm - 2.00 mS/cm
Solubility (1.5% solution @ OD <sub>640</sub> nm)	Minimum 95% Transmittance
Heavy Metals	< 10 ppm
Arsenic	< 2 ppm
Standard Plate Count	< 10 CFU/gram
Yeast/Mold	< 10 CFU/gram
Activity (Shugar units)	Minimum 23,500 units/mg
M <sub>r</sub>	14,600
EC Number	3.2.1.17
CAS Number	[12650-88-3]
Extinction Coefficient	E <sub>281.5</sub> <sup>1%</sup> = 26.4 (Anue and Tanford 1969)
Isoelectric Point	pH 11.0 (Alderton et al. 1945)

### Inhibitors

The enzyme is inhibited by surface-active reagents such as dodecyl sulfate, alcohols, and fatty acids (Smith and Stoker 1949). Imidazole and indole derivatives are inhibitors via formation of charge-transfer complexes (Shinitzky et al. 1966; Swan 1972).

### Stability

Lysozyme stored as a dry lyophilized or crystalline powder at 2 - 8°C is stable for years. Solutions at pH 4 - 5 are stable for several weeks refrigerated and for 24 to 48 hours at ambient temperatures. Sterile filtration of the sample solution after formulation and storage in a sterile container will enhance stability.

### Function

Lysozyme (muramidase) hydrolyzes preferentially the  $\beta$ -1,4 glucosidic linkages between N-acetylmuramic acid and N-acetylglucosamine which occur in the mucopeptide cell wall structure of certain microorganisms, such as *Micrococcus lysodeikticus*. A somewhat more limited activity is exhibited towards chitin oligomers (Holler et al. 1975 a and b).

Recommended solubilization buffer for crystallization is 0.02 M Sodium acetate trihydrate pH 4.6. Solubilization is slow and complete at room temperature up to 100 mg/ml. Vigorous shaking or vortexing will denature the sample. Suspend the sample into the sample buffer at room temperature and gently rock. The sample may be centrifuged to sediment amorphous or insoluble material or the sample may be filtered using a 0.22 - 0.45 micron filter.

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### Sample Concentration for Crystallization

20 to 100 mg/ml (Low sample concentrations typically produce fewer, larger crystals than high sample concentrations). The outcome of the experiment also depends on the composition and concentration of the reagent used for crystallization.

### Suggested Lysozyme Crystallization Reagent Conditions

Mix equal amounts of solubilized lysozyme with each of the following reagents and perform either a microbatch, free interface diffusion or vapor diffusion crystallization experiment. If precipitation is present, reduce the concentration of the lysozyme stock and repeat the experiment.

- a) 0.6 to 1.1 M Sodium chloride,  
0.1 M Sodium acetate trihydrate pH 4.2 to 4.8
- b) 0.6 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.2 to 4.8,  
25% Ethylene glycol
- c) 0.6 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.2 to 4.8,  
25% Glycerol
- d) 30% w/v Polyethylene glycol monomethyl ether 5,000,  
1.0 M Sodium chloride, 50 mM Sodium acetate trihydrate pH 4.5
- e) 5% w/v Sodium chloride,  
0.1 M Sodium acetate trihydrate pH 4.2 to 4.8

### Unit Definition

Shugar unit. Using *Micrococcus lysodeikticus*, one unit of lysozyme will cause a decrease in absorbance at 450 nm of 0.001 per minute at 25 degrees Celsius.

### References

Alderton, G., Ward, W., and Febold, H.: Isolation of Lysozyme from Egg White, *J Biol Chem* 157, 43, 1945.

Aune, K., and Tanford, C.: Thermodynamics of the Denaturation of Lysozyme by Guanidine Hydrochloride. I. Dependence on pH at 25 deg., *Biochem* 8, 4579, 1969.

Davies, A., Neuberger, A., and Wilson, B.: The Dependence of Lysozyme Activity on pH and Ionic Strength, *Biochim Biophys Acta* 178, 294, 1969.

Holler, E., Rupley, J., and Hess, G.: Productive and Unproductive Lysozyme-Chitosaccaride Complexes. Equilibrium Measurements, *Biochem* 14, 1088, 1975.

Holler, E., Rupley, J., and Hess, G.: Productive and Unproductive Lysozyme-Chitosaccaride Complexes. Kinetic Investigations, *Biochem* 14, 2377, 1975.

Shinitzky, M., Katchalski, E., Grisaro, V., and Sharon, N.: Inhibition of Lysozyme by Imidazole and Indole Derivatives, *Arch Biochem Biophys* 116, 332, 1966.

Smith, G., and Stoker, C.: Inhibition of Crystalline Lysozyme, *Arch Biochem Biophys* 21, 383, 1949.

Swan, I.: The Inhibition of Hen Egg-White Lysozyme by Imidazole and Indole Derivatives, *J Mol Biol* 65, 59, 1972.

### Technical Support

Inquiries regarding Lysozyme and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

### Related Products

**HR7-108** Lysozyme Kit 12 x 20 mg

**HR7-110** Lysozyme 8 grams

**HR2-805** 30% w/v Polyethylene glycol monomethyl ether 5,000,  
1.0 M Sodium chloride, 0.05 M Sodium acetate trihydrate  
pH 4.6, 100 milliliters

**HR2-731** 1.0 M Sodium acetate trihydrate pH 4.6, 100 milliliters

**HR2-637** 5.0 M Sodium chloride, 200 milliliters

**HR2-219** Grid Screen Sodium Chloride kit

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