SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of LYSOZYME[1] (EC 3.2.1.17)

PRINCIPLE:

Micrococcus lysodeikticus Cells (Intact) $\xrightarrow{\text{Lysozyme}}$ Micrococcus lysodeikticus Cells (Lysed)

CONDITIONS: T = 25°C, pH = 6.24, A_{450nm}, Light path = 1 cm

METHOD: Turbidimetric Rate Determination

REAGENTS:

A. 66 mM Potassium Phosphate Buffer, pH 6.24 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.24 at 25°C with 1 M KOH.)

B. 0.015% (w/v) Micrococcus lysodeikticus Cell Suspension (Substrate)
(Prepare 25 ml in Reagent A using Micrococcus lysodeikticus, ATCC 4698 lyophilized cells, Sigma Prod. No. M-3770. The A_{450nm} of this suspension should be between 0.6 and 0.7.)

C. Lysozyme Enzyme Solution
(Immediately before use, prepare a solution containing 200 - 400 units/ml of lysozyme in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent B (Substrate)	2.50	2.50

Equilibrate to 25°C. Monitor the A_{450nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Enzyme Solution)	0.10	------
Reagent A (Buffer)	------	0.10

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PROCEDURE: (continued)

Immediately mix by inversion and record the decrease in A_{450nm} for approximately 5 minutes. Obtain the ΔA_{450nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{450nm}/\text{min Test} - \Delta A_{450nm}/\text{min Blank})(df)}{(0.001)\ (0.1)}$$

df = Dilution factor
0.001 = Change in absorbance at A_{450nm} as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

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

FINAL ASSAY CONCENTRATION:

In a 2.60 ml reaction mix, the final concentrations are 66 mM potassium phosphate, 0.014% (w/v) Micrococcus lysodeikticus cell suspension and 20 - 40 units lysozyme.

REFERENCE:

Shugar, D. (1952) *Biochimica et Biophysica Acta* **8**, 302-309

NOTES:

1. This assay procedure is not to be used to assay Lysozyme, Bovine Recombinant Expressed in Pichia pastoris, Sigma Prod. No. L-9772, Lysozyme, Human, Recombinant Expressed in Pichia pastoris, Sigma Prod. No. L-2026, and Lysozyme Insoluble Enzyme on Agarose, Sigma Prod. No. L-1129.

NOTES: (continued)

2. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.