

Enzymatic Assay of LYSOSTAPHIN

PRINCIPLE:

Staphylococcal Cells (intact) $\xrightarrow{\text{Lysostaphin}}$ Staphylococcal Cells (lysed)

CONDITIONS: T = 37°C, pH 7.5, $A_{620\text{nm}}$, Light path = 1 cm

METHOD: Turbidimetric Rate Determination

REAGENTS:

- A. 50 mM Tris HCl Buffer with 145 mM Sodium Chloride, pH 7.5 at 37°C
(Prepare 1 liter in deionized water using Trizma Base, Sigma Prod. No. T-1503 and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 7.5 at 37°C with 1 M HCl.)
- B. Cell Suspension¹
(Prepare using the following steps:
 1. Prepare 100 ml of a 30% (w/v) Tryptic Soy Broth in Reagent A using Tryptic Soy Broth, Sigma Prod. No. T-8261. In a 250 ml flask, inoculate 50 ml of the Tryptic Soy Broth with 1 ml of a 1 mg/ml culture of *Staphylococcus aureus*,² American Type Culture Collection #29737. Incubate, with gentle shaking, at 37°C for 18 - 24 hours.
 2. Centrifuge the culture to pellet the cells. Remove the supernatant liquid and suspend the cells in cold Reagent A. Repeat the centrifugation and resuspend the cells in a minimum volume of cold Reagent A.
 3. Adjust the cell density to an $A_{620\text{nm}}$ of 1.3 - 1.5 by diluting with cold Reagent A. Keep the diluted suspension on ice until ready to assay samples.

THE FINAL DILUTED SUSPENSION (STEP 3) MUST BE PREPARED FRESH. USE THE STEP 3 CELL SUSPENSION WITHIN 15 - 20 MINUTES.)

- C. Lysostaphin Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.25 unit/ml of Lysostaphin in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	-----	5.00
Reagent C (Enzyme Solution)	5.00	-----

Equilibrate to 37°C for approximately 5 minutes. Then add:

Reagent B (Cell Suspension)	1.00	1.00
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Mix by swirling and immediately determine at zero time, (T_0), the A_{620nm}^3 , for both the Test and the Blank using Reagent A as a reference.

Incubate the cuvette at 37°C for exactly 10 minutes. Mix by swirling and determine at ten minutes, (T_{10}), the A_{620nm} for both the Test and the Blank.

CALCULATIONS:

$$r A_{620nm} = A_{620nm} \text{ Test} - A_{620nm} \text{ Blank}$$

$$\% \text{ Lysis} = \frac{r A_{620nm} T_0 - r A_{620nm} T_{10}}{r A_{620nm} T_0} \times 100\%$$

$$\text{Units/ml} = \frac{r A_{620nm} T_0 - r A_{620nm} T_{10}}{(0.125)(5)} \text{ (df)}$$

df = Dilution factor

5 = Volume (in milliliters) of enzyme assay

0.125 = The change in turbidity produced by 1 unit of
of Lysostaphin (as per the Unit Definition)

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

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

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UNIT DEFINITION:

One unit will reduce the turbidity ($A_{620\text{nm}}$) of a suspension of *S. aureus* cells from 0.250 to 0.125 in 10 minutes at pH 7.5 at 37°C in a 6.0 ml reaction mixture.

FINAL ASSAY CONCENTRATION:

In a 6.00 ml reaction mix, the final concentrations are 50 mM Tris, 145 mM sodium chloride and 0.25 - 1.25 units lysostaphin.

REFERENCE:

Iversen, O.-J. and Grov, A. (1973) *Eur. J. Biochem.* **38**, 293-300

NOTES:

1. Reproducibility of this assay is very dependent on the freshness and quality of the cell suspension.
2. This procedure incorporates the use of a potentially infectious microorganism. Containment and/or disinfection of all such products, solutions and test reagents is imperative. Heat or chemical sterilization of all contaminated reagents and any spills or releases, with subsequent disposal as Biohazardous Waste, must be employed.
3. The $A_{620\text{nm}}$ at zero time (T_0) should fall between 0.2 - 0.3 absorbance unit.
4. This assay is based on the cited reference.
5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.