

Enzymatic Assay of MUTANOLYSIN

PRINCIPAL:

Streptococcus faecalis Cell Walls (Insoluble) $\xrightarrow{\text{Mutanolysin}}$ Soluble Products

CONDITIONS: T = 37°C, pH = 6.0, A_{600nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM MES Buffer with 1 mM Magnesium Chloride pH 6.0 at 37°C (Assay Buffer)
(Prepare 50 ml in deionized water using MES, Sigma Prod. No. M-8250 and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 6.0 at 37°C using 1 M NaOH.)
- B. 50 mM TES with 1 mM Magnesium Chloride pH 7.0 at 37°C. (Enzyme Diluent)
(Prepare 50 ml in deionized water using TES, Sigma Prod. No. T-1375 and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 7.0 at 37°C using 1 M NaOH.)
- C. *Streptococcus faecalis* Cell Wall Suspension¹
(Substrate Solution)
(A cell wall suspension of log-phase *Streptococcus faecalis* STF-3 (ATCC 12784) cells in Reagent A are diluted to an A_{600nm} of 0.48 to 0.52 using Reagent A.)
- D. Mutanolysin Enzyme Solution
(Immediately before use, prepare a solution containing 100 - 200 units per ml in cold Reagent B.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent C (Substrate Solution)	3.00	3.00

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PROCEDURE: (continued)

Equilibrate at 37°C and monitor the $A_{600\text{nm}}$ until constant using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Solution)	0.05	-----
Reagent B (Enzyme Diluent)	-----	0.05

Immediately mix by inversion and record the decrease in $A_{600\text{nm}}$ for 20 minutes. Obtain the $\Delta A_{600\text{nm}}/\text{min}$ using the maximum linear rate for both the Test and Blank.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{600\text{nm}}/\text{min Test} - \Delta A_{600\text{nm}}/\text{min Blank})(3.05)(\text{df})}{(0.01)(0.05)}$$

3.05 = Volume (in milliliters) of reaction mix

df = Dilution factor

0.01 = Decrease in absorbance per minute as defined by the unit definition

0.05 = 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 per ml will produce a $\Delta A_{600\text{nm}}$ of 0.01 per minute at pH 6.0 at 37°C using a suspension of *Streptococcus faecalis* cell walls as substrate.

FINAL ASSAY CONCENTRATION:

In a 3.05 ml reaction mix, the final concentrations are 49 mM MES, 1 mM MgCl_2 , 0.82 mM TES, and 5 - 10 units mutanolysin.

REFERENCE:

Calandra, G.B. and Cole, R.M. (1980) *Infection and*

Immunity **28**, 1033-1037

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NOTES:

1. The preparation of Streptococcal cells for the assay of mutanolysin is described in the Sigma Chemical Co. Microbiology department protocol MQ-045.
2. This assay is based on the cited reference.
3. 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.