Enzymatic Assay of MUTANOLYSIN

PRINCIPAL:

Streptococcus faecalis Cell Walls (Insoluble) → 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 and Magnesium Chloride, Hexahydrate.
   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 and Magnesium Chloride, Hexahydrate.
   Adjust to pH 7.0 at 37°C using 1 M NaOH.)

C.  Streptococcus faecalis Cell Wall Suspension
    (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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Substrate Solution)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>
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**PROCEDURE:** (continued)

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent B (Enzyme Diluent)</td>
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Immediately mix by inversion and record the decrease in $A_{600nm}$ for 20 minutes. Obtain the $\Delta A_{600nm}/\text{min}$ using the maximum linear rate for both the Test and Blank.

**CALCULATION:**

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

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 $\Delta A_{600nm}$ 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:**

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NOTES:

1. The preparation of Streptococcal cells for the assay of mutanolysin is described in the Chemical Co. Microbiology department protocol MQ-045.

2. This assay is based on the cited reference.

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

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