



SIGMA-ALDRICH

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of MUTANOLYSIN

PRINCIPAL:

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

CONDITIONS: T = 37°C, pH = 6.0, $A_{600\text{nm}}$, 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. M8250 and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M0250. 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. T1375 and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M0250. 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 $A_{600\text{nm}}$ 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

Enzymatic Assay of MUTANOLYSIN

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

Enzymatic Assay of MUTANOLYSIN

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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.