



SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of ACHROMOPEPTIDASE (EC 3.4.21.50)

PRINCIPLE:

Micrococcus lysodeikticus Cells (intact) $\xrightarrow{\text{Achromopeptidase}}$ Micrococcus lysodeikticus Cells (lysed)

CONDITIONS: T = 37°C, pH 8.0, A_{600nm}, Light path = 1 cm

METHOD: Turbidimetric

REAGENTS:

- A. 10 mM Tris HCl Buffer with 10 mM Sodium Chloride, pH 8.0 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503 and Sodium Chloride, Sigma Prod. No. S-9888. Adjust to pH 8.0 at 37°C with 1 M HCl.)
- B. Micrococcus lysodeikticus Cell Suspension (Substrate)
(Prepare 30 ml in Reagent A by resuspending Micrococcus lysodeikticus, Sigma Prod. No. M-3770, to a cell density which produces an absorbance of 0.60 at A_{600nm} using a suitable spectrophotometer.)¹
- C. Achromopeptidase Enzyme Solution
(Immediately before use, prepare a solution containing 350 - 700 units/ml of Achromopeptidase in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	-----	0.10
Reagent B (Substrate)	2.90	2.90

Mix by inversion and equilibrate to 37°C. Then add:

Reagent C (Enzyme Solution)	0.10	-----
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PROCEDURE: (continued)

Immediately mix test and the blank by inversion and record the decrease in $A_{600\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{600\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{600\text{nm}}/\text{min Test} - \Delta A_{600\text{nm}}/\text{min Blank})(3.0)}{(0.001)(0.1)}$$

3.0 = Volume (in milliliters) of assay

0.001 = Change in absorbance at 600 nm (Unit Definition)

0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will produce a $\Delta A_{600\text{nm}}$ of 0.001 per minute per ml at pH 8.0 at 37°C using a suspension of *Micrococcus lysodeikticus* (M-3770) as substrate.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 10 mM Tris, 10 mM sodium chloride, suspended cells of *Micrococcus lysodeikticus* (producing an absorbance of 0.600 at $A_{600\text{nm}}$) and 35 - 70 units achromopeptidase.

REFERENCE:

Ezaki, T. and Suzuki, S. (1982) *Journal of Clinical Microbiology* **16**, 844-846

NOTES:

1. This value is approximately equal to 0.19 mg/ml.

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NOTES: (continued)

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

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