

Enzymatic Assay of PENICILLIN AMIDASE¹
(EC 3.5.1.11)

PRINCIPLE:

Benzylpenicillin + H₂O $\xrightarrow{\text{Penicillin Amidase}}$ 6-APA + Phenylacetic Acid

Abbreviation used:

6-APA = 6-Aminopenicillanate

CONDITIONS: T = 37°C, pH = 7.8

METHOD: Titrimetric

REAGENTS:

- A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 37°C
(Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)
- B. 2.0% (w/v) Penicillin-G Solution (PEN-G)
(Prepare 100 ml in Reagent A using Penicillin-G, Sodium Salt, Sigma Stock No. PEN-NA.)
- C. 500 mM Sodium Hydroxide Solution - Standardized (NaOH)
(Prepare 100 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.²)
- D. 100 mM Potassium Phosphate Buffer, pH 7.5 at 37°C
(Enz Dil)
(Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)
- E. Penicillin Amidase Enzyme Solution
(Immediately before use, prepare a solution containing 30 - 40 units/ml of Penicillin Amidase in cold Reagent D.)

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PROCEDURE:

Using a suitable pH meter in conjunction with a magnetic stirrer, pipette (in milliliters) the following reagents into a suitably thermostatted titration vessel:

	<u>Test</u>
Reagent B (PEN-G)	20.00

Equilibrate to 37°C. Then add:

Reagent E (Enzyme Solution)	1.00
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Immediately adjust to pH 7.8 with Reagent C (NaOH) using a burette.

Monitor the pH of the reaction mix. Record the time when the pH reaches 7.8. Maintain the pH of the reaction mix at pH 7.8 by the addition of small volumes (50 µl) of Reagent C. Record the volume of Reagent C used to maintain the pH at 7.8 and the time required.

CALCULATIONS:

(NaOH) = Volume (in milliliters) of Reagent C used in the Test required to maintain the pH at 7.8.

$$\text{Units/ml protein} = \frac{(\text{NaOH})(M)(1000)(df)}{(T)(1)}$$

M = Molarity of Reagent C

1000 = Conversion factor from millimoles to micromoles

df = Dilution factor

T = Time (in minutes) required to maintain the pH at 7.8

as per the Unit Definition

1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of benzylpenicillin per

minute at pH 7.8 at 37°C.

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INITIAL ASSAY CONCENTRATION:

In a 21.00 ml reaction mix, the initial concentrations are 52 mM potassium phosphate, 1.9% (w/v) penicillin-G, and 30 - 40 units penicillin amidase.

REFERENCES:

(1993) *Reagent Chemicals ACS Specifications*, 8th ed., **95**

Abraham, E.P. (1955) *Methods in Enzymology*, II, 120 - 120

NOTES:

1. This assay is not to be used to assay Penicillin Amidase, Insolubilized Enzyme, attached to macroporous oxirane acrylic beads (O-7628), Sigma Prod. No. P-3942.
2. Standardization of NaOH is described in (1993) *Reagent Chemicals ACS Specifications*.
3. This assay is based on the cited references.
4. 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.