

Enzymatic Assay of PENICILLIN AMIDASE Insolubilized Enzyme

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 Sodium Phosphate Buffer, pH 7.8 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751.)
- B. 4.0% (w/v) Penicillin-G Solution (PEN-G)
(Prepare 10 ml in Reagent A using Penicillin-G, Procain Salt, Sigma Stock No. PEN-P.)
- 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.¹)
- D. Penicillin Amidase Insolubilized Enzyme Suspension
(Prepare by suspending 200 mg of Penicillin Amidase, Insolubilized Enzyme, attached to macroporous oxirane acrylic beads (07628) in 20 ml of Reagent A. Filter through a 0.45 μm filter. Resuspend the filtered insoluble enzyme in 20 ml of Reagent A.)

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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>	<u>Blank</u>
Reagent D (Enzyme)	20.00	-----
Reagent A (Buffer)	-----	20.00

Mix by stirring at 37°C for 30 minutes. Then add:

Reagent B (Pen-G)	1.00	1.00
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Immediately adjust to pH 7.8 with Reagent C (NaOH) using a burette.

Run the reaction for 1 – 5 minutes. 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 (0.05 ml) 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 required to maintain the pH at 7.8.

$$\text{Units/g enzyme} = \frac{[(\text{NaOH}) \text{ Test} - (\text{NaOH}) \text{ Blank}](M)(1000)}{(T)(0.2)}$$

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

0.2 = Weight (in g) of solid penicillin amidase insolubilized enzyme used in the assay

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of benzylpenicillin per minute at pH 7.8 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 21.00 ml reaction mix, the initial concentrations are 50 mM potassium phosphate, 1.9% (w/v) penicillin-G, and 200 mg penicillin amidase insolubilized enzyme.

REFERENCES:

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

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

NOTES:

1. Standardization of NaOH is described in (1993) *Reagent Chemicals ACS Specifications*.
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.