

**Enzymatic Assay of PENICILLINASE
(EC 3.5.2.6)
Benzylpenicillin as Substrate**

PRINCIPLE:

Benzylpenicillin + H₂O $\xrightarrow{\text{Penicillinase}}$ Benzylpenicilloic Acid

CONDITIONS: T = 25°C, pH = 7.0

METHOD: Titrimetric

REAGENTS:

- A. 100 mM Tris HCl Buffer with 0.1% (w/v) Bovine Serum Albumin, pH 7.0 at 25°C
(Prepare 50 ml in deionized water using Trizma Base, Prod. No. T-1503, and Albumin, Bovine, Prod. No. A-4503 or equivalent. Adjust to pH 7.0 at 25°C with 1 M HCl.)
- B. Benzylpenicillin Solution (B-Pen)
(Immediately before use, prepare 500 ml of a solution containing 200 - 300 units/ml in deionized water using Penicillin-G, Sodium Salt, Stock No. PEN-NA.)
- C. 10 mM Sodium Hydroxide Solution-Standardized (NaOH)
(Prepare 400 ml in cold deionized water using Sodium Hydroxide, Anhydrous, Stock No. 505-8. Standardize according to the ACS Reagent Procedure.¹)
- D. Penicillinase Enzyme Solution
(Immediately before use, prepare a solution containing 3 - 7 units/ml of Penicillinase in cold 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 B (B-Pen)	50.00	50.00

Adjust to pH 7.0 at 25°C with Reagent C (NaOH), using a burette. Then add:

Reagent A (Buffer)	-----	0.10
Reagent D (Enzyme Solution)	0.10	-----

Monitor the pH of the reaction mix. Record the time when the pH reaches 7.0. The time for the test procedure is 10 minutes. Maintain the pH of the reaction mix at pH 7.0 by the addition of small volumes (50 µl) of Reagent C. Record the volume of Reagent C used to maintain the pH at 7.0 and the time required.

CALCULATION:

[NaOH] = Volume (in milliliters) of Reagent C used in the Test minus the Blank.

$$\text{Units/mg enzyme} = \frac{[\text{NaOH}] \text{ (M) (1000)}}{\text{(T) (mg enzyme/RM)}}$$

M = Molarity of Reagent C

1000 = Conversion factor from millimoles to micromoles (Unit definition)

T = Time required to maintain the pH at 7.0 (in minutes) as per unit definition

RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of benzylpenicillin to benzylpenicilloic acid per minute at pH 7.0 at 25°C.

INITIAL ASSAY CONCENTRATIONS:

In a 50.10 ml reaction mix, the initial concentrations are 0.2 mM Tris, 10,000 - 15,000 units benzylpenicillin, 0.0002% (w/v) BSA and 0.3 - 0.7 unit penicillinase.

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REFERENCES:

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

Abraham, E.P. (1955) *Methods of Enzymology*, Volume II, 120

NOTES:

1. Standardization of NaOH is described in the cited reference.
2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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