

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

**PRINCIPLE:**

Cephaloridine + H<sub>2</sub>O  $\xrightarrow{\text{Penicillinase}}$  Hydrolyzed Cephaloridine

**CONDITIONS:** T = 25°C, pH = 7.0, A<sub>255nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 100 mM Potassium Phosphate Buffer, pH 7.0 at 25°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod.No. P-5379. Adjust to pH 7.0 at 25°C with 1 M KOH.)
- B. 1 mM Cephaloridine Solution (Ceph)  
(Prepare 10 ml in Reagent A using Cephaloridine, Sigma Prod.No. C-3519.)
- C. Penicillinase Enzyme Solution  
(Immediately before use, prepare a solution containing 5 - 10 Cephaloridine units/ml of Penicillinase in cold Reagent A.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.70	2.71
Reagent B (Ceph)	0.30	0.30

Mix by inversion and equilibrate to 25°C. Monitor the A<sub>255nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

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**PROCEDURE:** (continued)

	<u>Test</u>	<u>Blank</u>
Reagent C (Enzyme Solution)	0.01	-----

Immediately mix by inversion and record the decrease in  $A_{255\text{nm}}$  for approximately 5 minutes. Obtain the  $r A_{255\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r A_{255\text{nm}}/\text{min Test} - r A_{255\text{nm}}/\text{min Blank})(3.01)(\text{df})}{(8)(0.01)}$$

3.01 = Volume (in milliliters) of assay

df = Dilution factor

8 = Change in the millimolar extinction coefficient between cephaloridine and the hydrolyzed  $\beta$ -lactam ring of cephaloridine

0.01 = 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 will hydrolyze 1.0  $\mu\text{mole}$  of cephaloridine per minute at pH 7.0 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 3.01 ml reaction mix, the final concentrations are 100 mM potassium phosphate, 0.1 mM cephaloridine and 0.05 - 0.1 cephaloridine unit of penicillinase.

**REFERENCES:**

Ross, G.W., and O'Callaghan, C.H. (1975) *Methods in Enzymology* **43**:80-81.

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**NOTES:**

1. 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.**