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TECHNICAL INFORMATION

Catalog Number: 190034
beta-Lactamase

Source: *Bacillus cereus*

E.C. # 3.5.2.6

Synonyms: Penicillinase; Penicillin amido-b-lactamhydrolase; Cephalosporinase

Activity: Each vial contains approximately 500 units beta-lactamase I and 50 units of beta-lactamase II

Unit Definition: One unit is defined as the amount of enzyme which will hydrolyze 1.0 micromole of benzyl penicillin and 1.0 micromole of cephalosporin C per minute at 25°C in the presence of EDTA, pH 7.0 and Zn^{2+}. (one unit is approximately equal to 600 Levy or 75 Pollock units).

Description: b-Lactamase is typically used to destroy b-lactam antibiotics.

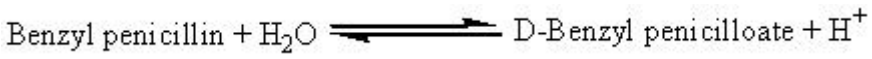
Inhibitors: Clavulanic acid, sulbactam, tazobactam. These inhibitors bind to the enzyme more effectively than the b-lactam antibiotic.

b-Lactam Antibiotic Effectiveness: Effective against benzylpenicillin, ampicillin, amoxycillin, carbenicillin, methicillin, cloxacillin, flucloxacillin.

Cephalosporin Antibiotic Effectiveness: Effective against cephaloridine, cephradine (above 100 ug/ml requires a 30 minute incubation to ensure complete inactivation), cephalothin, cephalexin, cefuroxime (requires a 24 hour incubation with an elevated concentration of enzyme), cephazolin, cefoxitin (requires a 24 hour incubation with an elevated concentration of enzyme). It is recommended that a control solution of the cephalosporin antibiotic is prepared and incubated under the same conditions as the sample. After incubation, the control should be tested for complete inactivation of the antibiotic.

Typical Assay Method:

beta-Lactamase I catalyzes the following reaction:



Reagents:

A.Benzyl Penicillin Solution: contains 4.5 uM benzyl penicillin, 0.35% gelatin and 5 mM EDTA prepared in double distilled, deionized water.
B.0.05 M NaOH solution
C.Enzyme Solution: Prepare enzyme in 0.1 M Tris-HCl, pH 7.0 with 1% gelatin.

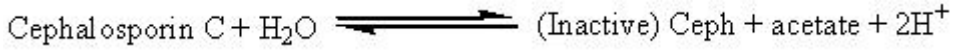
Method:

Use 2.0 ml benzyl penicillin solution (Reagent A). Equilibrate and titrate to pH 7.0 with 0.05 M NaOH at 25°C. Add 0.02 ml enzyme solution. Determine titration rate at 25°C using 0.05 M NaOH.

Calculation:

$$u/ml = \frac{V/min \times concentration\ NaOH}{sample\ volume}$$

beta-lactamase II catalyzes the following reaction:



Reagents:

A.Cephalosporin C Solution: containing 3.3 mM Cephalosporin C with 0.35% gelatin prepared in double distilled, deionized water.
B.0.1 M $ZnSO_4$ Solution in water.
C.0.05 M NaOH Solution.
D.Enzyme Solution: Prepare enzyme in 0.1 M Tris-HCl, pH 7.0 with 1% gelatin.

Method:

Use 2.0 ml Cephalosporin C Solution (Reagent A). Add 0.01 ml $ZnSO_4$ Solution (Reagent B). Equilibrate and titrate to pH 7.0 with 0.05 M NaOH at 25°C. Add 0.1 ml enzyme solution (Reagent D). Determine titration rate at pH 7.0, at 25°C with 0.05 M NaOH.

Calculation:

$$\text{u/ml} = \frac{\text{V/min x concentration NaOH}}{\text{2 x sample volume}}$$

Typical Method of Use:

Cultures:

Cultures are routinely prepared in order to test for bacterial infection. False negative results might be obtained where the samples contain antibiotics. Incorporation of b-lactamase in the culture medium will overcome this problem when penicillins or cephalosporins are present.

– Reconstitute the contents of one vial of b-lactamase (containing approximately 500 units b-lactamase I and 50 units b -lactamase II) in 5 ml sterile water.
– Add 1 ml b-lactamase solution to 100 ml culture medium.
– Incubate cultures and nutrient broth at 37°C for 18-24 hours or according to preferred protocol.

Effectiveness:

Dilution of the antibiotics in culture medium coupled with the long incubation time ensure effective inactivation of all the penicillins and cephalosporins listed above, even at concentrations higher than those normally encountered in serum.

Assay of Aminoglycoside Antibiotics

Aminoglycoside antibiotics such as gentamicin are commonly used in combination with penicillins and/or cephalosporins. Assay of the potentially nephrotoxic aminoglycosides is simplified by removal of b-lactam antibiotics from the sample. b-Lactamase is capable of inactivating a wide range of penicillins and cephalosporins thereby allowing assay of aminoglycoside antibiotics by microbiological methods.

– Reconstitute the contents of one vial of b-lactamase (containing approximately 500 units b-lactamase I and 50 units b -lactamase II) in 5 ml sterile water.
– Add 0.2 ml enzyme solution to 1.0 ml serum or cerebrospinal fluid.
– Incubate at room temperature for 5 minutes.
– Use sample in assay method of preference.

Alternatively, where the sample is too small to permit accurate dilution with enzyme, the b-lactamase can be incorporated into the assay medium as follows:

– After autoclaving and addition of test organism, add 0.2 ml b-lactamase solution to 10 ml tripticase soy agar.
– Mix thoroughly and pour suspension into Petri dishes or glass tubes.
– Storage at 4°C is possible for up to one week without significant loss of b-lactamase activity.

The alternative method where b-lactamase is incorporated into the assay medium is effective for all the penicillins listed above and for cephaloridine. The recommended method for inactivation of the other cephalosporins is by incubation of the sample with

b-lactamase before assay.

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