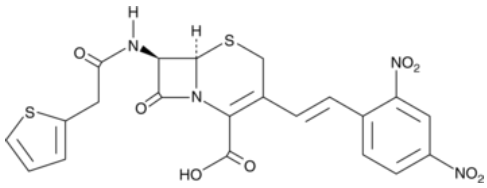
Cyrusbioscience

Nitrocefin



Product No.: 101-41906-86-9

CAS No.: 41906-86-9

Package: 25mg

Formula: $C_{21}H_{16}N_4O_8S_2$

M.W.: 516.50

Stability: ≥ 2 years at -20℃

Synonym:3-(2,4-Dinitrostyryl)-(6R,7R)-7-(2-thienylacetamido)-ceph-3-em-4-carboxylicAcid; Nitrocephin

Introduction:

Beta-Lactamases (βLs), are a large family of hydrolases comprising more than 850 identified members expressed in Gram-positive and Gram-negative bacteria. βLs can be classified according to their substrate or inhibitor specificity. These enzymes are capable of hydrolyzing four atom rings known as β-lactams. Antibiotics containing β-lactam rings (i.e. penicillin, cephalosporin, monobactam, carbapenem) are highly susceptible to be hydrolyzed via enzymatic activity, which deactivates their antibiotic potency. βLs have become a significant clinical threat due to the alarming number of cases of bacterial strains showing β-lactam antibiotic resistance. Nitrocefin offers a simple and sensitive assay that can detect and quantify the enzymatic activity of these hydrolases.

Description:

Nitrocefin is the chromogenic cephalosporin that acts as an excellent β-lactamase substrate. It exhibits a rapid distinctive color change from yellow (max at pH 7.0 = 390 nm) to red (max at pH 7.0 = 486 nm) as the amide bond in the beta-lactam ring is hydrolyzed by a β-lactamase. It is sensitive to hydrolysis by all known lactamases produced by Gram-positive and Gram-negative bacteria.

$$\text{Nitrocefin (Yellow)} \xrightarrow{\text{Beta-Lactamase}} \text{Product (Red) (OD486nm)}$$

Application:

Measurement of β-Lactamase activity in various biological samples

Protocol:

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1. Direct Plate Method

Add one drop of the Nitrocefin working solution on to the surface of the colony. If the isolate is a high β-lactamase producer then the colony and the surrounding area will turn red quickly.

2. Slide Method

Add one drop of the Nitrocefin working solution on to the surface of a clean glass slide. Using a sterile loop, pick one colony from the plate and emulsify into the Nitrocefin drop. Report as positive for β-lactamase if the color changes from yellow to red within 30 min.

NOTE: Protect the slide from desiccation during the waiting period.

3. Broth Method

Add four drops of the Nitrocefin solution to 1 ml of the grown culture. Report as positive for β-lactamase if the color changes to red within 30 min.

4. Broken Cell Method

Sonicate 1 ml of the culture in order to break open the cells. Add 4 drops of the Nitrocefin working solution. Report as positive for β-lactamase if the color changes to red within 30 min.

5. Paper Disc Spot Method

Place a Whatman No. 1 filter paper disc (diameter 7 cm) in a petri dish and impregnate with 5 ml of the Nitrocefin working solution. Apply an isolated colony to the impregnated paper disc using a sterile loop. A pink to red reaction developing within 15 minutes indicates the presence of β-lactamase.

NOTE: The impregnated paper disc is stable for one day, if protected from light to avoid degradation.

6. Spectrophotometric Assays for Determining β-Lactamase Activity

The working solution of Nitrocefin (500 µg/ml) is diluted ten-fold in buffer (0.1 M phosphate; 1 mM EDTA, pH 7.0). Spectrophotometric assays for β-Lactamase using Nitrocefin are carried out by measuring changes in absorbance at 486 nm. The molar extinction coefficient of hydrolyzed Nitrocefin at 486 nm is 20,500 M-1 cm-1. Test samples of the finished product for performance with control cultures.

Storage: Store at -20℃.

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