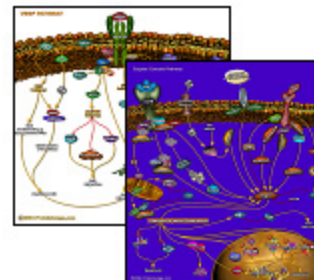


## Nitrocefin Cat. No. 484400



*Note that this data sheet is not lot-specific and is representative of the current specifications for this product. Please consult the vial label and the certificate of analysis for information on specific lots. Also note that shipping conditions may differ from storage conditions. Full details are available at [www.calbiochem.com](http://www.calbiochem.com).*

**Alternate Names:** 3-(2,4-Dinitrostyryl)-(6R, 7R)-7-(2-thienylacetamido)-ceph-3-em-4-carboxylic Acid, E-isomer

**Size:** 1 mg  
10 mg

**Description:** A chromogenic  $\beta$ -lactamase substrate that undergoes distinctive color change from yellow ( $\lambda_{\max} = 390$  nm at pH 7.0) to red ( $\lambda_{\max} = 486$  nm at pH 7.0) as the amide bond in the  $\beta$ -lactam ring is hydrolyzed by  $\beta$ -lactamase. Nitrocefin is sensitive to hydrolysis by all known lactamases produced by Gram-positive and Gram-negative bacteria. Also useful for the detection of  $\beta$ -lactamase patterns from bacterial cell extracts by isoelectric focusing. Has been used in competitive inhibition studies in developmental work on  $\beta$ -lactamase-resistant antibiotics.

### Recommended reaction conditions:

#### Techniques for the Rapid Detection of $\beta$ -Lactamase Using Nitrocefin

##### 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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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 Nitrocefim 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  $\beta$ -lactamase.

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

## 6. Spectrophotometric Assays for Determining $\beta$ -Lactamase Activity

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

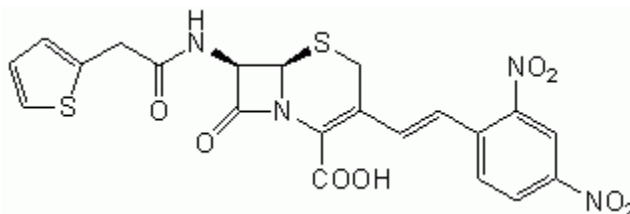
**Form:** Orange-yellow solid. Packaged under inert gas.

**CAS Number** 41906-86-9

**Molecular Weight:** 516.5

**Molecular Formula:**  $\text{C}_{21}\text{H}_{16}\text{N}_4\text{O}_8\text{S}_2$

**Structure:**



**Purity:**  $\geq 95\%$  by UV

**Solubility:**

### Preparing a Nitrocefim (500 $\mu\text{g/ml}$ ) Solution

- Dissolve 1 mg Nitrocefim in 100  $\mu\text{l}$  dimethylsulfoxide (DMSO) and vortex.
- Add 1.9 ml phosphate buffer (100 mM, pH 7) to produce 2 ml total volume.
- This yields a working Nitrocefim solution of 500  $\mu\text{g/ml}$  (approx. 1 mM), which is suitable for most applications.
- Nitrocefim, particularly in solution, is very sensitive to light.

**Storage:** FREEZER ( $-20^\circ\text{C}$ ). Protect from light. Following reconstitution, aliquot and freeze ( $-20^\circ\text{C}$ ). Stock solution may be stored at  $-20^\circ\text{C}$  for up to 2 weeks.

**Toxicity:** MSDS available upon request.

**References:**

- Guay, R., et al. 1980. *IRCS Med. Science* **8**, 209.  
King, A., et al. 1980. *Antimicrob. Agents Chemother.* **17**, 165.  
Matthew, M., et al. 1975. *J. Gen. Microbiol.* **88**, 169.  
O'Callaghan, C.H., et al. 1972. *Antimicrob. Agents Chemother.* **1**, 283.

**USA and Canada**  
Tel (800) 628-8470  
technical@calbiochem.com

**Germany**  
Freephone 0800 100 3496  
techservice@merckbiosciences.de

**United Kingdom and Ireland**  
UK Freephone 0800 622935  
Ireland Toll Free 1800 409445  
techservice@merckbiosciences.co.uk

**All Other Countries**  
**Contact Your Local Distributor**  
www.calbiochem.com  
technical@calbiochem.com

A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany  
www.calbiochem.com

**FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.**