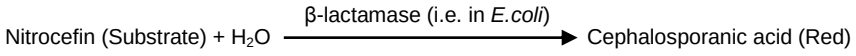
BugDisc™ Nitrocefin Disc

4/17

(Catalog # M1251-50; 50 discs; Store at -20°C)

I. Introduction:

Beta-Lactamases (βLs), are hydrolases expressed in Gram-positive and Gram-negative bacteria. When those bacteria encounter β-lactam antibiotics which contain the four atom ring-structure, they secrete βLs and hydrolyze these compounds. 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 are a significant clinical threat because of the bacterial strains with β-lactam antibiotic resistance. Resistance in gram-negative organisms may be caused by chromosomally or plasmid-mediated β-lactamases, and there is an increase in antibiotic resistance in bacteria in both community infections or hospital acquired infections. BioVision's Nitrocefin Discs offer a simple and sensitive assay that can detect the presence of β-lactamase enzymatic activity. The qualitative detection test is based on the hydrolysis of Nitrocefin, a yellow-colored β-Lactamase substrate impregnated into a filter paper disc. Upon incubation with β-lactamase producing bacteria and cleavage of Nitrocefin, the product cephalosporanic acid is produced and turns the filter paper red. A negative result is indicated by a lack of color change on the filter paper. The test is simple, requires no additional reagents and will yield reliable results in 5 minutes or less!



II. Applications:

- Determination of β-lactamase presence through cleavage of the substrate Nitrocefin.

III. Sample Types:

- Bacteria such as *E.coli*, enterococci, *N. gonorrhoeae*, or *H. influenzae*

IV. Kit Contents:

Components	M1251-50	Cap Code	Part Number
BugDisc™ Nitrocefin Disc	50	WM	M1251-50-1

V. User Supplied Reagents and Equipment:

- Glass slide, inoculating loop, wooden applicator stick
- dH_20
- Bacteria strains with/without β-lactamase

VI. Storage Conditions and Reagent Preparation:

This product is ready for use and no further preparation is necessary. Store product in its original container at -20°C. For short-term storage, this product can be stored at 4°C for 1 week. Before use, allow disc to reach Room Temperature (RT). Read the entire protocol before performing the assay.

VII. Beta Lactamase Assay Protocol:

1. **Sample Preparation:** Grow bacteria overnight on nonselective media such as LB or Mueller Hinton.
2. **Remove Nitrocefin Disc** and place on glass slide. Allow disc to acclimate to RT. Add 1 drop (10 µl) of dH_2O to Nitrocefin Disc.
3. **Remove ~five bacteria colonies from culture** with a sterile inoculating loop, pipette tip, or wooden applicator stick. Smear bacteria onto Nitrocefin Disc. Alternatively, use a swab to sweep the plate to obtain growth from a bacteria isolate and wipe onto disc.
4. **Incubate** the disk from 1-15 minutes at room temperature to observe color change.

Note: Positive reactions may take up to 60 minutes to develop for some staphylococci.

VIII. Measurement

1. **Observe** disc for a color change to pink or red (See time course below for *E.coli).*

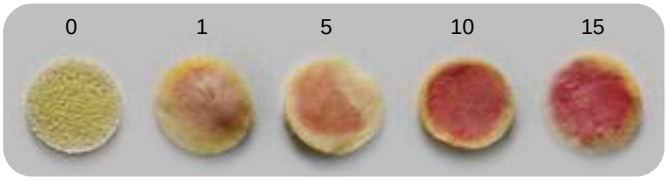


Figure 1: Time course. Each disc is impregnated with Nitrocefin. Upon incubation of the disc with *E.coli* and cleavage of the yellow-colored substrate, a red product is formed. The red color can be produced (yielding a positive result) in as little as 1 minute. The time course above indicates the increased amount of color as accumulation of cleaved substrate increases..

IX. Related Products

Beta-lactamase inhibitor Screening Kit (Colorimetric) (K804)
Beta-lactamase Activity Colorimetric Assay Kit (K803)
EZScreen™ Beta-Lactamase Activity Colorimetric Assay Kit (384-well) (K954)
Nitrocefin (2388)

FOR RESEARCH USE ONLY! Not to be used on humans.