

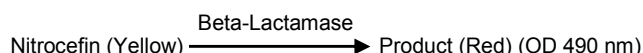
Beta-Lactamase Activity Colorimetric Assay Kit

(Catalog # K803-100; 100 assays; Store at -20°C)

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I. Introduction:

Beta-Lactamases (β LS), are a large family of hydrolases comprising more than 850 identified members expressed in Gram-positive and Gram-negative bacteria. β LS 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. β LS have become a significant clinical threat due to the alarming number of cases of bacterial strains showing β -lactam antibiotic resistance. BioVision's Beta-Lactamase Activity Assay Kit offers a simple and sensitive assay that can detect and quantify the enzymatic activity of these hydrolases. The assay is based on the hydrolysis of Nitrocefin, a chromogenic cephalosporin, that results in the generation of a colored product (OD 490 nm), which is directly proportional to the amount of β L activity. The assay can detect enzymatic activity as low as 0.06 mU in a variety of biological samples.



II. Application:

- Measurement of β -Lactamase activity in various biological samples
- Analysis of β -Lactamase activity in pathological conditions

III. Sample Type:

- Serum, urine, saliva from mammals infected with β L-secreting bacteria
- Food (e.g. milk)
- Fermentation media, bacterial cultures, etc.

IV. Kit Contents:

Components	K803-100	Cap Code	Part Number
β L Assay Buffer	27 ml	WM	K803-100-1
Nitrocefin (in DMSO)	220 μ l	Blue	K803-100-2
Positive Control (Lyophilized)	1 vial	Green	K803-100-3
β L Hydrolysis Buffer	100 μ l	Purple	K803-100-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- DMSO

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **β L Assay Buffer and β L Hydrolysis Buffer:** Warm β L Assay Buffer and β L Hydrolysis Buffer to room temperature before use.
- **Nitrocefin (in DMSO):** Warm to room temperature before use. Store at -20°C. Use within two months.
- **Positive Control:** Reconstitute with 20 μ l β L Assay Buffer. Mix well. Aliquot & store at -20°C. Avoid repeated freeze/thaw. Stable for two months.

VIII. Beta-Lactamase Assay Protocol:

1. Sample Preparation: Liquid samples (i.e. biological fluids, fermentation media) can be assayed directly. Collect bacterial samples by centrifugation (10000 x g; 10 min.) in a pre-weighed centrifuge tube. Remove supernatant and determine wet weight of the pellet. Resuspend the pellet in β L Assay Buffer using a minimum of 5 μ l of β L Assay Buffer per mg of sample. Sonicate samples for 5 min. Keep samples on ice for 5 min. Remove insoluble material by centrifugation at 16000 x g at 4°C for 20 min. Collect the supernatant. Add 1-50 μ l of supernatant into desired well(s) in 96-well plate. Adjust the volume to 50 μ l/well with β L Assay Buffer. For Positive Control, dilute Positive Control 5-fold by adding 2 μ l Positive Control to 8 μ l of β L Assay Buffer. Add 1-10 μ l of diluted Positive Control into desired well(s). Adjust the volume to 50 μ l/well with β L Assay Buffer.

Note:

For unknown samples, we suggest doing a small pilot experiment & testing several doses to ensure the readings are within the Standard Curve linear range.

2. Standard Curve Preparation: Hydrolyze Nitrocefin stock solution using β L Hydrolysis Buffer and DMSO (1:2:7) by adding 4 μ l of Nitrocefin, 8 μ l of β L Hydrolysis Buffer and 28 μ l of DMSO (not provided) in an eppendorf tube. Incubate the reaction at 60°C for 10 min. Cool down the reaction to room temperature and briefly centrifuge the tube. Add 0, 2, 4, 6, 8 & 10 μ l of the hydrolyzed Nitrocefin Standard (2 mM) into a series of wells in a 96-well plate to generate 0, 4, 8, 12, 16 & 20 nmol/well of Nitrocefin Standard. Adjust the volume to 100 μ l/well with β L Assay Buffer.

Note: Prepare hydrolyzed Nitrocefin solution fresh every time. Discard unused hydrolyzed Nitrocefin.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
βL Assay Buffer	48 µl
Nitrocefin	2 µl

Mix well. Add 50 µl of the Reaction Mix to the wells containing samples and Positive Control(s).

4. Measurement: Measure the absorbance (OD 490 nm) kinetically at room temperature for 30-60 min., protected from light.

Note: Incubation time depends on the beta-Lactamase activity in samples. Longer incubation times may be required if sample's βL activity is low.

We recommend measuring the OD in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the beta-lactamase activity of the samples. The Nitrocefin Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time [60 min.]).

5. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the Nitrocefin Standard Curve. Calculate the βL activity of the test sample: $\Delta OD = A_2 - A_1$ at a linear region of the curve. Apply the ΔOD to the Nitrocefin Standard Curve to get B nmol of hydrolyzed Nitrocefin generated by βL during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{Sample } \beta\text{L Activity} = B / (\Delta T \times V) \times D = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** is the amount of Nitrocefin from the Standard Curve (nmol)

ΔT is the reaction time (min.)

V is the sample volume added into the reaction well (ml)

D is the sample dilution factor

βL Activity can also be expressed as mU/mg of protein.

Unit Definition: One unit of βL activity is the amount of enzyme that generates 1.0 µmol of Nitrocefin per min. at pH 7.0 at 25°C.

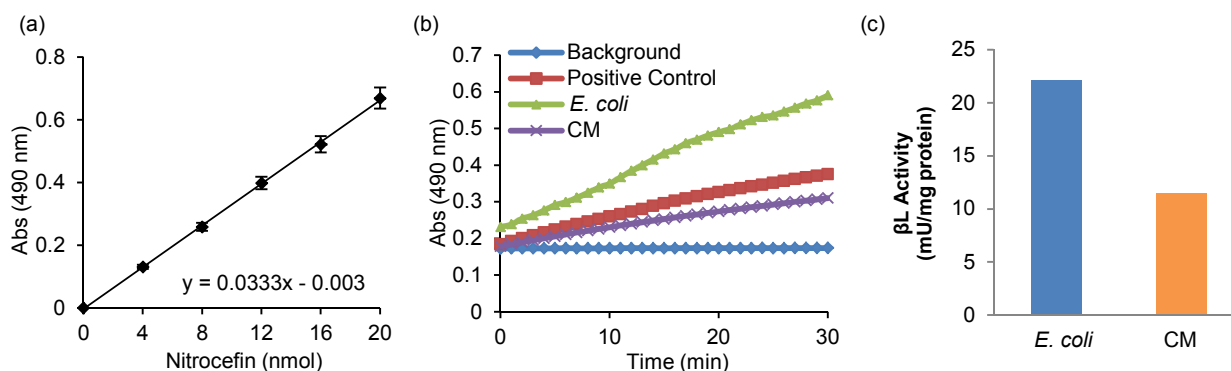


Figure: a) Nitrocefin Standard Curve. b) βL activity in *E. coli* culture (5 µl), contaminated media (CM; 30 µl) & Positive Control (4 µl). c) βL Activity of *E. coli* and contaminated media expressed per milligram of protein. Assay was performed following the kit protocol.

IX. Related Products:

CENTA β-Lactamase Substrate (2394)

Nitrocefin (2388)

Beta-Lactamase Inhibitor Screening Kit (Colorimetric) (K804-100)

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